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Soil Science and Agrochemistry

DANGERS OF ARSENIC DRINKING AND IRRIGATION WATER TO PLANTS AND HUMANS. ANTAGONISM OF ARSENIC AND MAGNESIUM

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The arsenic content of the irrigation water accumulates through absorption in the top soil and, thus concentrated, inhibits the development of plants, reducing their volume of yield. We studied the effects of various concentrations of arsenic on barley, onion and rice plants grown in soil and culture fluid. Arsenic inhibited the growth and production of the plants in every case. Magnesium (fertilizer), given simultaneously with arsenic, inhibited the uptake of arsenic, and so prevented its damage. In the groundwaters of the southern regions of Trans-Tisza, the arsenic content is relatively high (0.1—0.3 mg/l). The arsenic content of water in driven (artesian) wells decreases with the well depth. The arsenic content of vegetal food and drinking water accumulates in the human and animal organism and decreases the efficiency of the immune system, the phagocytic activity of macrophages. Arsenic and magnesium are antagonistic to one another (according to our findings), so the uptake and toxicity of arsenic can be reduced by magnesium application.

Keywords: antagonism, arsenic, artesian water, barley, immune system, irrigation water, magnesium, man (human), onion, rice

Introduction

Arsenic is a widespread element of the biosphere; in the soil generally 1—40 mg/kg arsenic can be found. However, when arsenic plant protectives are used, and further, in the neighbourhood of chemical works, coal-fed power plants, and in the case of irrigation water containing arsenic, its concentration may be essentially higher. In the flue dust of coal-fed power plants, 50—500 mg/kg arsenic can be found, depending on the origin of the coal, which is a considerable amount, since the yearly amount of dust discharge can be measured in thousands of tons per power plant.

Minimum quantities of arsenic can usually be found in plants. An average plant seldom contains arsenic at concentrations higher than 1 mg/kg (ppm), unless it comes from an area contaminated by arsenic. In most human foodstuffs only a few tenths ppm of arsenic can be found in terms of fresh weight. The arsenic content of algae and of foods and feeds of marine origin may be much higher; e.g. in eels, while generally 6—8 mg/kg, it may even reach 100 mg/kg.

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In the organism of an adult human, 15—20 mg arsenic (0.2—0.3 mg/kg) can generally be found; the average arsenic content of the blood is 0.12 mg/kg. The keratinous skin, nail and hair have a relatively high arsenic content. An average hair contains 0.5 mg/kg arsenic, while a hair with higher than 3 mg/kg arsenic-content suggests the possibility of arsenical poisoning. The arsenic content of cow's milk is 0.05 mg/l (Pais, 1980).

According to some data, arsenic promotes growth in tissue cultures, and it has shown a favourable effect on the growth and feed conversion of young pigs. The most recent literary data give an account of the carcinogenic effect of arsenic.

On the role of arsenic in plant organisms, rather conflicting statements can be found. Most of the data support its toxicity. We also experienced the toxic effect.

The reason for carrying out analyses was given us by the relatively high (0.1—0.3 mg/kg) arsenic-content in the drinking and irrigation water of ground-water origin in the southern regions of Trans-Tisza. The arsenic content of the irrigation water is absorbed and accumulates in the top-soil, thus inhibiting the growth of plants, and decreasing the volume of yield. Moreover, through the nutrition chain, it may even be harmful to the human organism.

Materials and methods

Our crop production experiments were carried out on slightly acid (pH = 6.0) sandy chernozem soil in 5 kg culture pots with 5 repetitions. Beside the uniform NPK rates, the soils were given arsenic ($NaAsO_2$) and magnesium ($MgSO_4$) treatments. The treatments were: control, 50 mg/kg arsenic, and 50 mg/kg arsenic + 500 mg/kg magnesium supplements. As indicator plants, spring barley of 'Soartan' variety and 'Makói' onion were used. In the case of onion, the above indicated arsenic soil treatment was substituted for by an arsenic treatment of the same level, but applied through irrigation onto the surface of the plant. With this we wished to study the effect of the arsenic content of irrigation water as exerted through the leaf, in comparison to a mere soil treatment. In the other experiment series, the varietal reactivity was examined with rice plants grown in a culture fluid, though with the same treatments as above.

The effects of the various treatments were established through phenological observation and by the analyses of the plants. The arsenic and magnesium contents of the plants were determined after wet destruction in sulphuric acid-hydrogen-peroxide. The magnesium was determined by atomic absorption spectrophotometry and the arsenic with hydrid-developing atomic absorption technique. The arsenic content of the water samples was examined with the hydride-developing method.

Results

The plants responded with a less intensive development to the arsenic treatment. Their leaves showed reddish, yellowish discoloration. Magnesium supplementing lessened the damaging effect of arsenic. Arsenic uptake was reduced to 40—50%. Arsenic uptake through the leaf was higher than it was through the root, in the case of identical arsenic concentrations.

In Table 1, analyses data for barley and onion plants are shown. According to the evidence of the data, the arsenic treatment increased the arsenic content of the plants. While the arsenic treatment only checked the magnesium uptake to a lesser extent (by 10—20%), the magnesium supplement reduced the arsenic content by

Table 1

Changes in the arsenic- and magnesium contents of barley and onion plants in response to different treatments

Treatment			Barley				Onion			
			Seed		Straw		Bulb		Stalk, leaf	
Method	As	Mg	As	Mg	As	Mg	As	Mg	As	Mg
	mg/kg		mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%
To soil	0	0	0.044	0.122	0.117	0.227	0.02	0.175	0.192	0.443
	50	0	0.688	0.108	1.539	0.214	0.20	0.172	0.987	0.444
	50	500	0.509	0.116	1.520	0.237	0.15	0.198	0.834	0.481
$LSD_{5\%}$	—	—	0.022	0.005	0.009	0.008	0.02	0.010	0.026	0.011
Mg effect %	—	—	35	7	1	11	33	15	18	8
To leaf	50	—	—	—	—	—	0.45	0.175	1.990	0.443

an average of 30% in the fruit (seed, bulb), by 15% in the stalk and leaf, and hardly at all in the straw of barley. In the case of leaf treatment (irrigation) the arsenic-content of the plant was higher than when the same treatment was applied to the soil. The analyses also showed that the onion plant was more reactive to the arsenic treatment than the barley.

Table 2

Changes in the weight, arsenic- and magnesium-contents of rice plants as a function of treatment and variety

Variety	Control	As treated	As + Mg treated	$LSD_{5\%}$	Mg effect. %
Orysella					
Weight mg/plant	30.7	11.7	13.3	0.5	+14
As mg/kg	0.6	108.0	90.0	3.0	−16
Mg mg/kg	1470.0	940.0	3230.0	20.0	+244
Nucleorisa					
Weight mg/plant	23.1	6.4	10.6	0.9	+65
As mg/kg	6.9	192.0	78.0	5.0	−46
Mg mg/kg	2000.0	1100.0	2870.0	23.8	+160
Mutashali					
Weight mg/plant	22.5	8.1	9.6	0.6	+19
As mg/kg	1.8	204.0	172.0	4.2	−16
Mg mg/kg	1680.0	1270.0	4060.0	42.0	+219

The effect of arsenic and magnesium treatment on 3 varieties of rice can be seen in Table 2. The results of these experiments show that both the damaging effect of arsenic and the tolerance to it caused by magnesium depend not only on the species but also on the variety.

Table 3

Arsenic-content in driven wells of some settlements in southern Trans-Tisza

Settlement	As mg/l	Settlement	As mg/l
Dévaványa	0.055	Kétsoprony	0.15
Gyomaendrőd	0.050	Kamut	0.14
Kondoros	0.060	Kötegyán	0.15
Orosháza	0.050	Kertészsziget	0.15
Újkígyós	0.075	Murony	0.13
Kőröstarcsa	0.076	Méhkerék	0.13
Mezőberény	0.064	Nagyszénás	0.12
Okány	0.062	Sarkad	0.14
Békéscsaba	0.104	Szeghalom	0.14
Bélmegyer	0.130	Telekgerendás	0.17
Füzesgyarmat	0.160	Tarhos	0.17
Gádoros	0.140	Békés	0.18
Gerla	0.120	Bucsa	0.31
Gyula	0.100	Doboz	0.20
Szarvas	0.107	Sarkadkeresztur	0.26

As-content permitted by the WHO: 0.05 mg/l

Table 4

Arsenic-content of wells of various depth in some settlements

Settlement	Depth of well (filter place) m	As mg/l
Magyartés	70	0.14
	200	0.03
Makó	54	0.30
	316	0.21
	500	0.03
Orosháza	50	0.07—0.15
	100	0.04—0.12
	200	0.02—0.06
	300	0.01—0.03
Székkutas	79	0.14
	155	0.03

In Table 3 the arsenic-contents of waters in the southern settlements of Trans-Tisza are seen. The data show that some 47% of the wells in the settlements contain more than 0.05 mg/l arsenic, the quantity permitted by the WHO.

As seen in Table 4, the arsenic-content of wells close to each other (often at a distance of merely 50 m) is in inverse proportion to the well depth. This is particularly dangerous, because in many cases water from shallower wells is used for irrigation.

Conclusions

The measuring results prove that the arsenic content of the soil, and particularly of the irrigation water, poisons the plants, which can be checked by magnesium supplementing (fertilization). Arsenic accumulates first of all in the stalk and leaf, and less of it is contained in the fruit.

The similar inhibitory effect of magnesium on toxicity was indicated in our earlier experiments with aluminium and cadmium microelements (Kiss, 1987, 1991; Oncsik et al. 1989) in various plants (bean, rice). Inhibition by magnesium of toxication with aluminium, cadmium and arsenic microelements can be explained by antagonism. We also found that inhibition by magnesium only occurred when the magnesium was present at a concentration higher by an order of magnitude than the microelements concerned. We note here that Guiet-Bara (1990) described the cadmium-magnesium antagonism in the case of human *placenta villi*. This confirms our statement, and at the same time proves its general biological validity, probably in the case of arsenic as well.

The damaging effect of arsenic introduced with the drinking water in the human organism may be further increased by the arsenic content of vegetal food. The damage of arsenic done to humans was studied by Chien-Jen (1985) in the popula-organism may be further increased by the arsenic content of vegetal food. The damage of arsenic done to humans was studied by Chien-Jen (1985) in the population of Taiwan. He found that the increasing number of malignant tumorous diseases was connected with the arsenic content of the drinking water; among the consumers of the high arsenic content (0.35—1.14 mg/l) artesian waters, the number (proportion) of those deceased in tumorous diseases was on an average twice the number of consumers of waters containing less or even no arsenic (0.0—0.3 mg/l). In regions exposed to arsenic water the number of those deceased in tumorous diseases was, in 1968, 80% higher than among the population consuming arsenic-free water. Among those consuming waters not contaminated with arsenic, the number of tumorous death cases increased by 20%, while among consumers of arsenic waters by 40% in 14 years. The exposure of the different organs as a function of high-, low arsenic content- and mixed waters consumed is shown in Fig. 1. Further, the investigations revealed that among women there were 30—40% more tumorous death cases than among men. Similar results were obtained by Tsuda (1990) among the workers of an arsenic-processing plant in Toroku (Japan).

According to Fisher (1986) the higher susceptibility to diseases of those consuming arsenic drinking water is due to the fact that the arsenic decreases the phagocytic activity of macrophages. $NaAsO_2$ decreased the phagocytosis by 33% at 1 μg/ml, and by 69% at 2 μg/ml concentration. The decrease caused by arsenic in the phagocytic activity of macrophages is shown in Fig. 2. According to Labedzka (1989) this decrease in the phagocytic activity is due to the fact that the bactericidal mechanism of the macrophages relates to their active oxygen (H_2O_2, O_2^-) production, which is in inverse ratio to the arsenic-content of the medium and decreases by 70% even at an arsenic concentration of 1 μM.

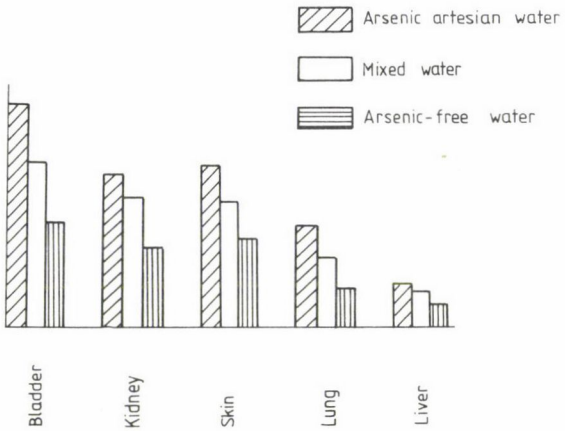

Fig. 1. Carcinogenic effect on human organisms of drinking waters with various arsenic-contents (Chien-Jen, 1985)

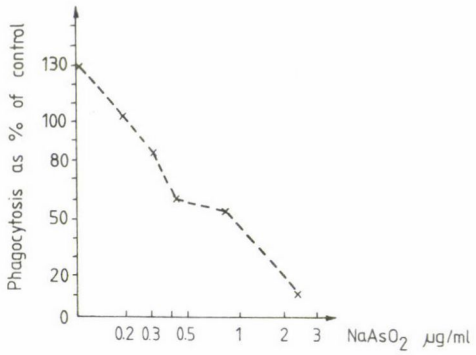

Fig. 2. Decrease in the phagocytic activity of macrophages as a function of arsenic-content (Fisher, 1986)

Accordingly, fertilizers containing magnesium, such as AGRONIT (2.5% Mg, 28% N) and KAMEX (2% Mg, 40% K_2O) are of higher value than other fertilizers which do not contain magnesium, even on 'arsenical' areas.

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Plant Physiology and Agrochemistry

SELF AND CROSS COMPATIBILITY OF HUNGARIAN SOUR CHERRY CULTIVARS

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Self-, open- and cross-pollination of 5 Hungarian sour cherry cultivars were studied in Helvécia, Hungary. The fruit set of artificially self-pollinated flowers was two to three times higher than that of naturally self-pollinated flowers. The self-pollination of uncaged flowers was more effective than artificial self-pollination.

'Pándy 7' and three Pándy-type self-fertile cultivars ('Debreceni bőtermő', 'Kántorjánosi 1' and 'Újfehértói fürtös') are considered to be genetically related to each other and partially interincompatible.

Keywords: cross-pollination, emasculation, fertilization, flower, flowering, self-fertile, self-sterile, sour cherry, variety

Introduction

New self-fertile, productive sour cherries have been derived in the last two decades by local selections and controlled hybridization, and these have been used to replace self-sterile, unproductive Pándy variety and other self-sterile cultivars. Maliga (1944, 1953), Brózik (1969) and Nyéki (1974) have discussed the compatibility of old sour cherry varieties. There are only a few publications on the compatibility of new varieties (Nyéki 1989; Nyéki and Szabó, 1990; Salgim, 1990). We have, therefore, made detailed studies on self- and cross-compatibility of some new cultivars which are most important in commercial orchards in Hungary.

Materials and methods

Sour cherry cultivars were studied between 1988 and 1990 in Helvécia in a collection of cultivars, situated in Central Hungary.

The trees were planted in 1981 at a spacing of 7×5 m. The fruit set of caged and artificially unpollinated flowers, caged and artificially pollinated flowers, open-pollinated flowers and cross-pollinated flowers were evaluated.

Self and cross compatibility of sour cherries were estimated annually on 100 to 400 flowers per cultivar. Uncaged branches were observed and results were compared to caged ones. Flowers were caged into paperbags in the early maturing stage.

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The fruit set of open-pollinated flowers was measured on a section of branches with 200—500 flowers at the medium height of the crown of the trees. The fruit set was evaluated 1 or 2 weeks prior to the ripening period.

The morphological characteristics were observed by studying 30 flowers per cultivar.

Results and discussion

The fruit set on bagged and unbagged branches

1. The fruit set of self-pollinated and open-pollinated flowers varied widely from year to year (Table 1).

Table 1

Fertility of sour cherry cultivars (Helvécia, 1988—1990)

Cultivars	Self-pollination (caged, artificially not poll.)				Fruit set (%) Artificial self-pollination				Open pollination (unbagged)			
	1988	1989	1990	Average	1988	1989	1990	Average	1988	1989	1990	Average
Cigánymeggy 7	6.8	17.4	32.7	19.0	16.7	34.5		25.6	21.4	37.4	55.8	38.2
Debreceni bőtermő	1.4	6.8	6.6	4.9	17.2	21.8		19.5	9.4	31.8	8.7	16.6
Kántorjánosi 1	5.2	6.7	4.9	5.6	10.2	10.8		10.5	17.4	37.1	12.8	22.4
Újfehértói fürtös	3.2	12.2	3.9	6.4	1.5	21.6		23.1	15.7	35.7	6.5	19.3
Pándy 7	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.1	7.0	27.0	15.3	16.4

2. Our previous (Nyéki, 1974) and recent work showed that none of the 'Pándy' clone's fruit set pollinated by their own or by other 'Pándy' clone's pollen. Their fruit set on unbagged branches was usually low.
3. 'Debreceni bőtermő', 'Kántorjánosi 1' and 'Újfehértói fürtös' varieties begin to ripen at the end of the maturity season of 'Pándy' clones. Their fruit quality is lower but closely similar to 'Pándy meggy'. Their self-compatibility features and their fruit set on unbagged branches are similar to each other.
4. Among the small-fruited, highly self-fertile 'Cigánymeggy' cultivars, 'Cigánymeggy 7' was chosen as the standard.
5. The fruit set of artificially self-pollinated flowers was two or three times higher than that of the naturally self-pollinated flowers in case of self-fertile cultivars. No increase was observed on self-sterile cultivars. The bee pollination on unbagged flowers was more effective than the artificial self-pollination.
6. 'Pándy 7' variety has been found to be self-sterile, and this cultivar has produced the lowest percentage of fruit set. There was a positive correlation between the rate of fruit set on self-pollinated flowers and on that of unbagged ones.

Cross-pollination

1. Pándy should be planted with pollinizers. The best pollinizers for the valuable 'Pándy' clone's were chosen by Brózik (1969) and Nyéki (1974).

2. The Pándy-type self-fertile cultivars ('Debreceni bőtermő', 'Kántorjánosi 1' and 'Újfehértói fürtös') are late blooming ones, like 'Pándy', but they are not suitable pollinizers for 'Pándy', according to our results (Table 2).

Table 2

Cross compatibility of sour cherry cultivars (Helvécia, 1988—1990)

♀ ♂		Pándy 7	Debreceni bőtermő	Kántorjánosi 1	Újfehértói fürtös	Cigánymeggy 7
Pándy 7	1988		0.0	0.0	0.0	30.5
	1989		0.0	0.0	20.8	30.6
	1990		0.0	0.0	0.0	2.0
Debreceni bőtermő	1989	8.2		14.3	15.2	—
	1990	3.2		2.5	1.9	1.0
Kántorjánosi 1	1989	6.3	—	—	—	—
	1990	0.0	0.0		1.6	2.8
Újfehértói fürtös	1989	1.8	—	—	—	—
	1990	0.0	0.0	2.3		4.4
Cigánymeggy 7	1990	9.8	2.6	1.6	0.0	

3. 'Pándy' was not a good pollinizer for any other Pándy-type cultivar, nor did these ones well pollinate each other. The fruit set of these combinations was low or changeable from year to year.
4. According to the fruit set in the case of cross-pollinations, 'Pándy' and other Pándy-type cultivars are considered to be partially interincompatible, but this statement needs further examination.

Table 3

Morphological characteristics of sour cherry flowers (Helvécia, 1989)

Cultivars	Number of flowers developed from a single flowerbud	Flower diameter (mm)	Number of stamens	Length of pistil (mm)
Pándy 7	4.3	33.0	30.3	12.0
Debreceni bőtermő	3.8	29.9	29.9	12.7
Kántorjánosi 1	4.2	30.1	30.1	11.8
Újfehértói fürtös	4.0	31.6	31.6	12.3
Cigánymeggy 7	2.6	24.7	32.6	10.4

Relations

Based on the morphological characteristics of the tree, the fruit and the flower (Table 3), 'Debreceni bőtermő', 'Kántorjánosi 1', 'Újfehértói fürtös' and 'Pándy 7' are considered to be genetically related to each other. These cultivars have similar types of flowers and their flower structure much differs from other varieties (e.g. 'Cigánymeggy 7').

Conclusions

The fruit set of self- and open-pollinated flowers varies from year to year. It is evident that self-sterile varieties should be planted with pollinizers. However, associated planting of sour cherries and the presence of honeybees during the blooming period also increase the fruit set in the case of self-fertile cultivars. Finally, it is important to stress that 'Pándy' and other Pándy-type sour cherry cultivars are unsatisfactory pollinizers for each other.

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IMPORTANCE OF NECTARIES IN THE FLOWER STRUCTURE OF PLUM CULTIVARS

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Interest in plum cultivars owing to both the cultivar research and the botanical problems of cultivars is justified in Hungary. Flowers from 45 cultivars were collected in Cegléd between 1986 and 1990, and the major morphogenetic characters were surveyed, with special emphasis on the colour of the stigma, anthers and nectary. Namely, the success of insect pollination and certain phylogenetic considerations give reason for these investigations.

The cultivars can be easily distinguished by the characters, the pistil length, the stamen number and even the petal median are highly significant. The correlation between petal median and relative stamen number ($r = -0.697$, $p = 1\%$), and between pistil length and stamen number ($r = -0.771$, $p = 1\%$) is proved; it became conspicuous in the course of grouping cultivars by self-fertility, and even more so when the grouping was based on the colour of the nectary.

Green nectary developed in those cultivars whose flowers have large petals, few stamina and low relative stamen number, respectively. Flowers with yellowish green and greenish yellow nectary were also significantly different from the orange-, yellow- and lemon-nectary group. The colour of the stigma was found to be less characteristic, with its ageing certainly occurred changes in its colour which could not be exactly followed. On the other hand, the colour of the anther is important, since from the point of view of the insects' (bees') orientation the colour has a stimulatory role.

Close correlation was found between the colour of the nectary and the stamen number ($r = +0.618$, $p = 1\%$), which confirmed the observations by Orosz-Kovács et al. (1989). The flower structure is thus important not only from the point of view of theoretical, morphological modelling, but also as a spectacular (colour) and nutritional (nectar) means of insect allurement.

Keywords: flower structure, morphogenetics, nectary, *Prunus*

Introduction

Ruellius (1543) was the first to describe the nectaries, Koelreuter (1761) already analysed nectar collected from flowers of many species. Linné (1763) wrote a review of nectaries published under the title "Nectaria florum". According to Sprengel (1793) nectar only occurs in insect-pollinated flowers, and the organs which produce nectar he called nectar glands.

Kurr (1933) having removed the pistils and stamina did not find any difference in the secretory activity and its chemical composition, although the modern examination have given a different result (cf. Durkee, 1983). Behrens (1879) and Bonnier (1879) summed up the knowledge of nectaries as early as in the last century, pointing out the diversity of their shapes and colours. Later Filarszky (1911), Brown (1938) and Ewert (1932) studied the nectaries from a taxonomic point of

view. Since Mercer and Rathgeber (1962) intensive studies have been carried out on the ultrastructure of the nectaries, because it influences the extent of mellification and the quality of the nectar alike.

Gulyás (1975) considered — with good reason — the colour of the nectary in the different species of the family *Labiatae* to be a phylogenetic character. The nectary of the earlier species is green, while that of the more developed ones is yellow, yellowish white or white. The function of the glands is also changing: from the radial those with zygomorphous symmetry developed.

The stone fruits can be traced back to a polyphyletic origin (Simmonds, 1976), therefore, the taxonomic place of the cultivars is very difficult to determine by a single aspect (cf. Kárpáti, 1967). The relationship between flower structure and fertilization (as a function) should be examined in a theoretical model (Surányi, 1980; 1985), and in a sense of pollination (Gulyás, 1975; Szabó et al., 1989) as well, since the amount of nectar, the periodicity of its production, its bitterness value, its sugar value and the pollen supply of the cultivars concerned — naturally together with the meteorological factors — exceedingly influence the movements of bees (McGregor, 1976).

The structure and surface formations of the *Prunoideae* are characteristic (Stace, 1965), the surface of the nectaries is striated, net-like, occasionally transitional (Orosz-Kovács et al., 1991); the adaxial side of the receptacle cavity is lined and cloak-like (Fahn, 1979). Similar diversity was found with the *Prunus* cultivars by Hungarian authors (Orosz-Kovács, Kovács, Gulyás, Kaposvári, 1989) the colour of the petals is important from both a phylogenetic and a fertilization point of view in the *Prunus* cultivars, which is of high significance if only because in the characteristics of the cultivars' flower structure important regularities have been found.

Between the morphogenetic characters (petal median, pistil length, stamen number, relative stamen number, stigma diameter, pollen germination) numerous correlations can be pointed out (Surányi, 1985), but the correlation between the stamen number and the colour of the nectary has but recently been proved (Orosz-Kovács et al., 1989). The inclination to self-fertility can be brought into connection both with the size of the nectary (which determines the extent of nectar production too) and with its composition (Májer-Bordács et al., 1989), which could be guessed from the examinations of Szabó et al., (1989) and from our own investigations (Surányi, 1980).

The importance of the stamen number (Morrison, 1964) can even be seen from Linné's taxonomic classification, as the size of flower, its sexual character, morphogenetic soundness and pollen production greatly influence the insect visitation, that is, they also have an indirect effect on fertilization. According to Stösser (1985) the bad pollen cultivars produce less and worse pollen than the good pollen sources of plum.

Further we think it necessary to examine how the colour of nectaries fits in the group of morphogenetic characters in the genus *Prunus*, since in the *Prunoidea* genera obvious trends can be observed (cf. Orosz-Kovács et al., 1990). We did not undertake to do more, because after our work in Cegléd the histological investigations in Pécs may result in the clearing up of further facts.

Materials and methods

Between 1986 and 1990 we examined 45 cultivars of the plum collection in Cegléd; each cultivar stood on myrobalan seedling, and the trees were 5—9 years old. Flowers of spurs at the beginning of anthesis were examined, 30 per cultivar. The mean value of the length and width of the petal, the pistil length, the stamen number, the relative stamen number and the stigma diameter were included in the survey. Further, we determined the colour of stigma, anther and nectary. The self-fertility value of the cultivars and the colour of the nectaries were regarded as an aspect of grouping too.

Seventeen self-fertile, 9 partially self-fertile and 19 self-sterile cultivars (Tóth and Surányi, 1980; Surányi, 1985), and 8 cultivars with green-, 19 with yellowish green–greenish yellow 8 and 18 with yellow and orange nectaries were included in our investigations (Table 1). With the view of a simpler analysis of the quality features we numbered the different shades of colour:

1 — green
2 — yellowish green
3 — orange green
4 — greenish yellow
5 — greenish orange
6 — orange, brownish orange
7 — yellow
8 — lemon-coloured

On the basis of the different morphogenetic characters the cultivars were statistically evaluated, and this was done according to the grouping. Earlier (Orosz-Kovács et al., 1989) we found a close correlation between the stamen number and the colour of the nectary, which was also analysed in this series of examination.

Results and discussion

The cultivars examined belong to the species *Prunus domestica, P. insititia* and *P. x italica*. The petal median ranged between 7.7 and 11.2 mm, only those of the virus infected trees showed wider fluctuation, but these were excluded from the evaluation. The fluctuation was 45% on an average, it was thus more than the difference in pistil size between the cultivars (2.5%). The average pistil length was 12.4 mm, ranging from 9.4 to 14.6 mm. Tuleu gras excelled with its very short pistil, opposed to the long pistils in the flowers of Ruth Gerstetter, President, Szakarka and Francia narancsszilva.

The difference in stamen number (17.3—32.3) was remarkably great between the cultivars; Beregi datolya, Ruth Gerstetter, Tuleu dulce and other differentiated less than 20 stamina, while Stanley, Bluefre and Golden sugar with their stamen number above 30 were also different from the others.

The results obtained for the relative stamen number were also highly significant, 1.20—2.65 stamina may even fall to a single millimetre length of pistil. The plum cultivars are included in Table 2 on the basis of the increasing values of the relative stamen number. The stigma diameter was below 800 μm for Beregi datolya, Tuleu dulce and Tuleu gras, and exceeded 1200 μm in the case of Ruth Gerstetter, Schüle korai and Szakarka (Table 2).

The correlation between the petal median and the relative stamen number ($r = -0.697$ and $p = 1\%$, as well as between the pistil length and the stamen number $r = -0.771$ and $p = 1\%$) was negative, but other correlations were not found between the characters.

Table 1

Colour of some flower parts and self-fertility of flowers in the cultivars examined

Cultivar	Fertility	Colour of neotary	Colour of stigma	Colour of anthers
Beregi datolya	PSF	green	yellow	lemon
Ruth Gerstetter	SS	green	yellow	lemon
Besztercei C.970	PSF	green	orange	orange
Schüle korai	SS	green	yellowish green	orange
Csahticka	SF	green	yellowish green	greenish yellow
Tuleu dulce	SS	green	green	orange
President	SS	green	greenish yellow	orange
Pescarus	SS	green	green	orange
Leppermann Emma	SF	yellowish green	orange	yellow
Gras ameliorat	SS	yellowish green	greenish yellow	yellow
Mammut Dorota	PSF	yellowish green	yellowish green	yellow
Jeruzsálemi kék	SS	yellowish green	yellowish green	lemon
Queckenboss	PSF	yellowish green	yellow	yellow
Centenar	SS	yellowish green	green	yellow
Albatros	SS	yellowish green	yellow	yellow
Tuleu timpuriu	SS	yellowish green	yellow	yellow
Augusztinka	PSF	yellowish green	yellowish green	orange
Čačanska II/II/80/59	SF	yellowish green	yellow	orange
De Soto	PSF	yellowish green	yellowish green	orange
Plovdivi csemege	SS	yellowish green	greenish yellow	yellow
Tuleu gras	SS	yellowish green	yellow	yellow
Óriás	PSF	yellowish green	yellowish green	yellow
Bavay	SF	yellowish green	greenish yellow	yellow
Čačanska rana	SF	yellowish green	yellowish green	yellow
Čačanska lepotica	SF	yellowish green	yellowish green	yellow
Sermina	SS	yellowish green	yellow	orange
Althann	SS	yellowish green	yellow	orange
Paczelt szilvája	SF	orange	greenish orange	yellow
Bühli korai	SF	brownish green	yellowish green	lemon
Szakarka	SF	greenish yellow	yellowish green	orange
Victoria	SF	brownish green	yellowish green	yellow
Francia narancsszilva	PSF	greenish orange	yellow	orange
Čačanska rodna	SF	greenish orange	yellow	yellow
Čačanska najbolja	SS	greenish yellow	yellow	orange
Ageni 707	SF	greenish yellow	greenish yellow	yellow
Wangenheim	SF	brownish green	greenish orange	lemon
VIR Vengerka	SF	greenish yellow	yellow	yellow
Ageni 698	SF	orange	yellow	yellow
Déli Vengerka	SF	greenish orange	yellow	greenish yellow
Utility	SS	greenish yellow	yellowish green	yellow
Bluefre	PSF	greenish orange	orange	orange
Stanley	SF	orange	greenish orange	yellow
Imperial Epineuse	SS	brownish green	yellow	lemon
Montfort	SS	greenish orange	greenish yellow	orange
Golden sugar	SS	greenish yellow	yellowish green	lemon

SF = self-fertile
PSF = partially self-fertile
SS = self-sterile

Table 2

Morphogenetic characteristics of the cultivars examined (1986—1990)

Cultivar	Petal median mm	Pistil length mm	Stamen number n	Relative stamen number n/mm	Stigma diameter μm
Beregi datolya	9.1	14.0	17.3	1.20	788
Ruth Gerstetter	9.2	14.6	18.5	1.31	1208
Besztercei C. 970	9.0	14.1	20.4	1.45	1192
Schüle korai	9.9	14.3	21.2	1.48	1212
Csahticka	9.4	13.4	20.6	1.69	1031
Tuleu dulce	9.0	10.0	17.6	1.70	799
President	9.4	14.5	25.7	1.79	1238
Pescarus	9.5	10.8	20.3	1.88	876
Leppermann Emma	8.9	12.9	20.3	1.59	1252
Gras amellorat	8.8	13.7	23.7	1.74	935
Mammut Dorota	8.8	13.0	23.3	1.76	1092
Jeruzsálemi kék	7.7	11.0	21.0	1.86	985
Queckenboss	8.7	12.7	23.3	1.87	1058
Centenar	8.1	10.0	18.8	1.89	1100
Albatros	8.7	10.6	20.0	1.89	888
Tuleu timpuriu	8.4	10.5	19.3	1.91	847
Augusztinka	8.6	12.9	25.3	1.93	1070
Čačanska II/II/80/59	8.4	12.2	24.0	1.94	877
De Soto	8.6	13.8	26.8	1.94	1180
Plovdivi csemege	8.6	13.2	26.1	1.98	1106
Tuleu gras	8.2	9.4	19.2	2.07	742
Óriás	7.9	11.4	26.4	2.22	934
Bavay	8.1	11.0	25.6	2.30	1022
Čačanska rana	8.2	11.9	27.5	2.37	962
Čačanska lepotica	8.3	11.7	28.2	2.41	980
Sermina	7.9	10.1	26.6	2.42	808
Althann	8.2	10.7	27.2	2.43	1077
Paczelt szilvája	9.0	13.8	21.1	1.50	1042
Bühli korai	8.1	12.2	19.2	1.55	879
Szakarka	8.6	14.5	27.1	1.86	1208
Victoria	8.5	13.0	24.4	1.87	1081
Francia narancs-szilva	11.2	14.4	29.1	2.00	933
Čačanska rodna	8.6	13.2	26.7	2.02	1136
Čačanska najbolja	8.5	12.3	26.0	2.11	1018
Ageni 707	8.5	12.5	28.2	2.13	1042
Wangenheim	11.2	12.6	27.3	2.18	1084
VIR Vengerka	8.3	12.2	26.7	2.20	1082
Ageni 698	8.4	12.7	27.9	2.23	1085
Déli Vengerka	8.4	11.8	27.1	2.25	950
Utility	8.0	12.2	29.2	2.37	1003
Bluefre	8.3	13.4	32.3	2.41	1125
Stanley	7.9	12.1	30.5	2.52	1085
Imperial Epineuse	8.0	11.3	28.9	2.55	923
Montfort	8.1	11.6	29.9	2.58	973
Golden sugar	9.0	12.1	32.0	2.65	877
$LSD_{5\%}$	0.39	0.31	0.56	0.24	62.4

Table 3 lists the characters according to fertility groups. Seventeen self-fertile, 9 partially self-fertile and 19 self-sterile plum cultivars did not represent the groups in such a measure as obtained in the previous years on the basis of 80 cultivars

(Surányi, 1985). This is the reason for the less than expected difference between the groups; still, the partially self-fertile and self-sterile plum cultivars significantly differed from one another (Table 3).

Table 3

Morphogenetic characteristics of the fertility groups

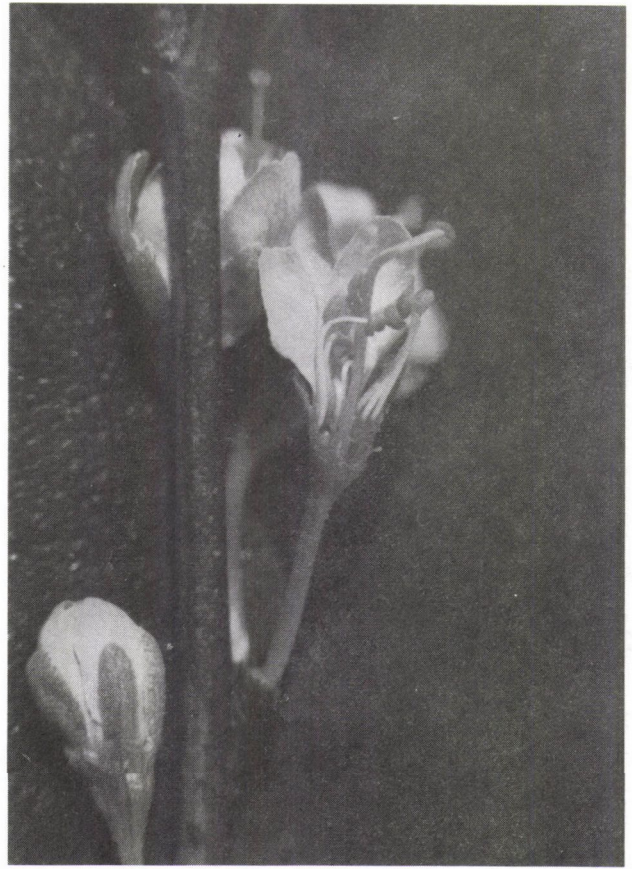
Characters	Self-fertile n=17	Partially self-fertile n=9	Self-sterile n=19	F-value
Petal median, mm	8.6	8.7	8.7	0.43
Pistil length, mm	12.6c	13.3c	11.3ab	7.96**
Stamen number, n	25.4	24.3	24.1	0.54
Relative stamen number, n/mm	2.01	1.76c	2.13b	1.16
Nectary colour	greenish-brownish yellow (3.5)bc	orange-green (2.0)a	orange-greenish (2.4)a	89.62***
Stigma colour	greenish-orange (4.8)	greenish-yellowish (4.5)	greenish-orange (4.4)	0.07
Stigma diameter, µm	1047	1062c	974b	2.13
Anther colour	orange (6.5)	orange (6.6)	yellow (6.8)	0.12

** p=1%
*** p=0.1%

The phylogenetic problem of the nectary colour was already mentioned in the Introduction; the C.970 Besztercei szilva and the Ageni 707 are the representatives of the green and greenish yellow nectaries; both are important cultivars, their apicultural importance is also great (Fig.1). The green nectary is associated with large petal, very few stamina and low relative stamen number, respectively. The difference compared to the other two is conspicuous according to the yellowish green — greenish yellow nectary group too. The colour of the stigma was also different for the green, yellow and orange nectaries. The stigma diameter was outstandingly small in the yellowish green — greenish yellow nectary group; while the colour of the anther was not a marked character. The Fisher test gave significant results, but the stamen number and the colour of the nectary coincided (Table 4).

The correlation mentioned by us (Orosz-Kovács et al., 1989) was now found confirmed for a different composition of cultivar too; it can be numerically proved that the (green) colour of the nectaries is associated with the (low) number of stamina, and the yellow and orange nectaries mostly can be observed in cultivars with large stamen number and high nectar production. According to the average of the 45 cultivars, the close correlation at 1% level is significant ($r = +0.618$) (Fig. 2).

In the last two decades the phylogenetic importance of the stamen number was studied in numerous experiments (Surányi and Tóth, 1976; 1977). We also found that the pistil length and the stamen number were in close correlation (Surányi, 1973), and the change of many other morphogenetic characters in the course of differentation was not by chance either, but they developed according to certain laws under the influence of competitive factors (Surányi, 1985; 1988).



a)

b)

Fig. 1. Flowers of plum-cultivars with green (C.970 Besztercei) and with orange nectary (Ageni 707)
(Photo: Zs. Orosz-Kovács)

Table 4

Characteristics of nectary colour groups

Characters	Green n=8	Yellowish-green-greenish yellow n=19	Yellow and orange n=18	F-value
Petal median, mm	9.3bc	8.4a	8.7a	5.66**
Pistil length, mm	13.2	11.7c	12.7b	4.69*
Stamen number, n	20.2bc	23.8ab	27.4ab	12.89***
Relative stamen number, n/mm	1.56bc	2.03a	2.16a	12.51***
Nectary colour	green (1.0)	yellowish green (2.0)	yellow-orange (4.4)	—
Stigma colour	greenish yellow (4.6)	yellow-greenish orange (3.8)	greenish orange (4.9)	0.69
Stigma diameter, µm	1043b	903a	1029	7.29**
Anther colour	orange (6.7)	yellow-orange (6.2)	yellow (6.8)	1.05

* p = 5%
** p = 1%
*** p = 0.1%

The flower structure as the result of differentiation, and the flower fertilization show close correlation, as the complex of the geno- and phenotypic characters. This is so (Surányi, 1976; 1980) as long as the conditions of fertilization are present in the flower, i.e. in the case of self-fertile and partially self-fertile cultivars. In the self-sterile cultivars, like with the insect-pollinated *Prunus*-es, the situation is different (Szabó et al., 1989; Brózik and Nyéki, 1975). As we pointed out in the foregoing, the properties of the nectaries, both their colour and nectar production greatly influence the possibilities of pollination.

The pollen- and nectar production of plum flowers are cultivar properties, though they are considerably influenced by environmental factors (Szabó et al.,

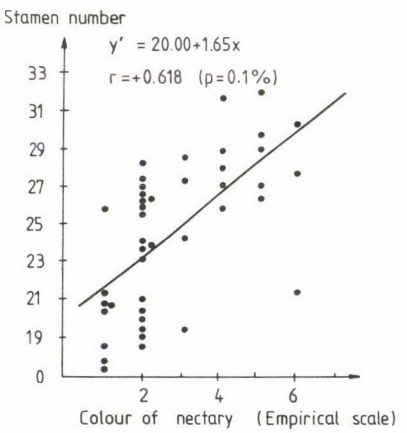


Fig. 2. Relation between nectary-colour and stamen number

1989); much pollen and high nectar sugar value are rather alluring. At the beginning of flowering pollen, then nectar is collected by the bees; of other flower-visiting insects we have fewer data (Péter, 1975; McGregor, 1976). The pollen production varies with cultivar, year and growing site alike, due to the stamen number and to meteorological conditions prevailing at the time when the previous year's flower buds differentiate (Tóth and Surányi, 1980).

According to Orosz-Kovács et al. (1990) in the *Prunus* species and cultivars the cuticle and the arrangement of stomata are specific features which influence the nectar production too; the fine structure of surface and the thickness of the nectary, in particular, are factors controlling the production. The phylogenetic aspects of flower structure (Surányi, 1980) and nectaries (Gulyás, 1975; Orosz-Kovács et al., 1990; 1991) are connected with one another, why, in the case of a culture species even the possible (selective) role of insects — some flowers are preferred, while other may be avoided by the bees — seems to be proved (cf. Kobel, 1954).

Insect visits to white-petalled plum flowers depend on the colour of the nectaries and the amount, and even on the taste of the nectar produced. As proved earlier (Péter, 1975), the nectar production of the plum cultivars is extremely varying, and for this very reason our investigations are not superfluous, not only from a botanical but from an economic (fertilization biology) point of view as well.

The cultivars included in the present study are potential cultivars of the cultivar change taking place even at present, so our experiences can be taken into consideration when planning the rows and association of cultivars in a new plantation. Namely, as we have pointed out, from the standpoint of bee visiting the amount of pollen per flower and the colour of the nectary also play some role, as they influence the actual volume of production.

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A STUDY OF POLLEN VIABILITY AFTER STORAGE AND THE EFFECT OF SOME GROWTH REGULATORS ON *IN VITRO* POLLEN GERMINATION AND POLLEN TUBE GROWTH IN *CICER*

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Pollen grains of *Cicer* remained viable (50%) for about 70 days depending upon the cultivars. There was a gradual decrease in viability from 0—75 days of pollen storage. The most suitable growth medium for *in vitro* pollen germination and pollen tube growth was standardised. In 0.5 M sucrose solution, all the cultivars exhibited a higher percentage of pollen germination and more pollen tube length. An addition of 100 ppm boric acid to this medium gave the best results. The effect of an addition of some growth regulators (Kn, ABA, IAA, GA) to the growth medium was studied on pollen germination and tube growth. In general, both these parameters were stimulated with all the growth regulators taken individually at 5 or 10 ppm concentration. With an increase in their concentration there was a negative effect. The presence of kinetin in the growth medium showed the best results, whereas abscisic acid seemed to be the least effective. The combination of kinetin and abscisic acid has no positive effect on pollen tube growth, though it increased pollen germination at lower concentrations. The combined effect of abscisic acid and IAA was positive in the least number of cases.

Keywords: *Cicer arietinum, C. reticulatum, C. judaicum, C. bijugum, C. cuneatum,* pollen, pollen tube

Introduction

Studies of pollen viability and longevity are important in understanding the problems of sterility in plants and hybridization studies, in order to provide pollen for the late-flowering varieties. The studies pertaining to pollen germination have received importance in recent years because of their role in the reproductive physiology of flowering plants. The low pollen germinability and slow rate of growth of the pollen tubes adversely affect the proper seed set in plants. Attempts have been made to overcome this problem with the judicious application of naturally occurring and synthetic plant growth substances by many investigators (Johri and Vasil, 1960; Patil and Rohman, 1976; Bisaria, 1974). Pollen has promoters and inhibitors which regulate pollen germination and tube growth (Stanley and Linskens, 1974; Malik and Bhandal, 1983). This study was done to standardise the most suitable medium for *in vitro* pollen germination and pollen tube growth, and to identify the individual and the combined effects of four growth regulators, i.e. Kinetin (Kn), Abscisic acid (ABA), Gibbrellic acid (GA) and Indole acetic acid (IAA) on the above mentioned parameters in *Cicer*.

Materials and methods

The experimental material used to standardise the most suitable medium for *in vitro* pollen germination and pollen tube growth comprised four cultivated varieties of *Cicer arietinum* of which two are 'desi' type, viz. C 235 and H 75-35 and two are of 'Kabuli' type viz. BG 315 and BG 422. To study the viability, pollen germination and tube growth of pollen after storage, and the effect of the growth regulators on pollen germination and pollen tube growth, the cultivated species *Cicer arietinum* var. C 235 and four annual wild species, i.e. *C. reticulatum, C. judaicum, C. bijugum* and *C. cuneatum* were taken.

Four concentrations of sucrose, i.e. 0.25 M, 0.5 M, 0.75 M and 1.0 M were tried for studying *in vitro* germination of pollen grains and tube growth after 4 hours in the growth medium. Since 0.5 M sucrose gave the best results, it was tried with four concentrations of boric acid, viz. 50 ppm, 100 ppm, 150 ppm and 200 ppm. The combination of 0.5 M sucrose and 100 ppm boric acid gave the best results and so it was used for further experiments. The percentage germination count was recorded from each slide prepared with pollen from five random flowers from one plant, and three random plants were selected from each species. From each slide, five random microscopic field were taken for determining the germination percentage. Tube length was determined by taking the average of ten longest tubes, i.e. two from each of the five random microscopic fields from each slide.

For pollen preservation fresh flowers were cut between 8-9 a.m. These were placed in a Petri dish and transferred to a desiccator and stored in a deep freeze at -4 °C. Pollen viability, pollen germination counts, and pollen tube growth measurements were made from 0—80 days at an interval of three days. For pollen viability studies pollen grains were stained with 2% acetocarmine and mounted in glycerine. The well filled and stained pollen grains were considered as viable and normal, and the unfilled, shrunken and unstained pollen grains were counted as unviable and aborted ones. For pollen germination counts and pollen tube growth, stored pollen was kept on the growth medium for 4 hours at 4 °C and the data was recorded.

The effect of growth regulators on *in vitro* pollen germination and tube growth was studied using five concentrations, i.e. 0, 5, 10, 15 and 20 μg/ml each of Kn, ABA, GA and IAA, separately and in combination. Hanging drop cultures were raised from the pollen incubated in the medium of 0.5 M sucrose, two percent agar (W/V) and 100 ppm boric acid at 24 ± 2 °C under diffused light (100—200 lux), as suggested by Bisaria (1986). For combination studies 10 μg/ml Kn was tried with various concentrations of IAA and GA separately. Also 10 μg/ml of ABA and various concentrations of IAA and GA and 10 μg/ml of IAA and various concentrations of GA were taken. The percentages of pollen germination and tube length were recorded on the basis of five random microscopic fields.

Results

The fresh pollen was stored in a desiccator at -4 °C and this pollen was used for such tests as pollen viability, pollen germination and pollen tube growth, by keeping it in a sucrose and boric acid medium. The most suitable concentration of sucrose and boric acid was standardised. In 0.5 M sucrose, all the varieties exhibited a higher percentage of germination and more tube length (Table 1). Variety BG 315 showed maximum pollen germination (69%) but the least pollen tube length (4.2 μ). Pollen tube length was maximum in C 235 (6.4 μ). The study was also done at different concentrations of boric acid, combined with 0.5 M sucrose, to select the best concentration of boric acid as a growth medium. At 100 ppm boric acid with 0.5 M sucrose, germination percentage was more than 80% and the tube length was maximum in all the varieties (Table 2). BG 422 had the maximum percent pollen germination (88.2%) but the longest pollen tube (10.4 μ) was recorded in BG 315. We selected this concentration of sucrose and boric acid for further studies.

The results of the pollen viability experiment indicated that, at 0 days of storage, fresh pollen showed the highest percent pollen fertility which was above

Table 1

Percent pollen germination and pollen tube growth (μ) of different varieties of Cicer arietinum in various concentrations of sucrose after 4 hours

Treatment	*C. arietinum* CV. C 235		*C. arietinum* CV. H 75—35		*C. arietinum* CV. BG 315		*C. arietinum* CV. BG 422	
	Germination (%)	Pollen tube length (μ)	Germination (%)	Pollen tube length (μ)	Germination (%)	Pollen tube length (μ)	Germination (%)	Pollen tube length (μ)
0.25 M Sucrose	44.2	2.6	42.0	2.4	38.0	2.8	40.0	2.2
0.50 M Sucrose	65.2	6.4	68.2	5.8	69.0	4.2	62.0	5.9
0.75 M Sucrose	58.5	4.8	46.5	3.2	48.5	3.4	52.5	3.6
1.0 M Sucrose	28.2	1.8	23.0	1.8	20.2	1.4	22.2	1.0
X	49.03	3.90	44.93	3.30	43.90	2.95	44.18	3.18
S. E.	±7.11	±0.91	±8.04	±0.76	±8.85	±0.51	±7.44	±0.91
$LSD_{5\%}$	15.06	1.92	17.03	1.61	18.75	1.08	15.78	1.93

Table 2

Percent pollen germination and pollen tube growth (μ) of different varieties of Cicer arietinum in sucrose (0.5 M) and various concentrations of boric acid after 4 hours

Treatment	*C. arietinum* CV. C 235		*C. arietinum* CV. H 75—35		*C. arietinum* CV. BG 315		*C. arietinum* CV. BG 422	
	Germination (%)	Pollen tube length (μ)	Germination (%)	Pollen tube length (μ)	Germination (%)	Pollen tube length (μ)	Germination (%)	Pollen tube length (μ)
0.5 M Sucrose + 50 ppm boric acid	74.0	9.4	65.0	8.4	78.2	9.8	72.2	7.8
0.5 M Sucrose + 100 ppm boric acid	86.0	10.2	82.2	9.8	84.2	10.4	88.2	9.6
0.5 M Sucrose + 150 ppm boric acid	70.2	7.4	68.2	8.2	68.0	7.8	70.0	6.8
0.5 M Sucrose + 200 ppm boric acid	64.2	6.8	62.2	6.2	59.0	5.8	66.2	5.6
$\bar{X}$	73.60	8.45	69.40	8.15	72.35	8.45	74.15	7.45
S. E.	±3.98	±0.69	±3.84	±0.64	±4.82	±0.90	±4.19	±0.73
$LSD_{5\%}$	8.44	1.47	8.15	1.36	10.21	1.92	8.89	1.55

90% (Fig. 1). With time there was a sequential decrease in pollen viability. After 75 days it decreased from 95 to 58% in C 235. Pollen was not available in species other than *C. arietinum* after 50 days of storage.

C. reticulatum showed the highest fresh pollen germination (85%) and *C. bijugum* the least (78%) (Fig. 2). The pollen tube was longest in C 235 (11.8 μ) and the shortest in *C. judaicum* (9.0 μ). The pollen stored in the desiccator showed a slight decrease in germination and tube growth on the 3rd day of storage. On the

5th day of storage of pollen, its germination came down from 82 to 32.5% in *C. arietinum*. Pollen tube also did not show much growth after 75 days of storage of pollen (Fig. 3).

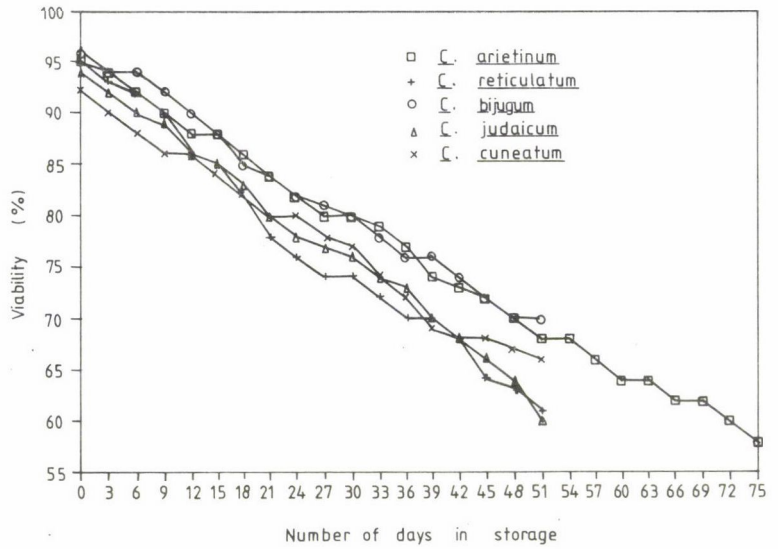

Fig. 1. Viability percentage of pollen grains of different species of *Cicer* after storage at −4 °C

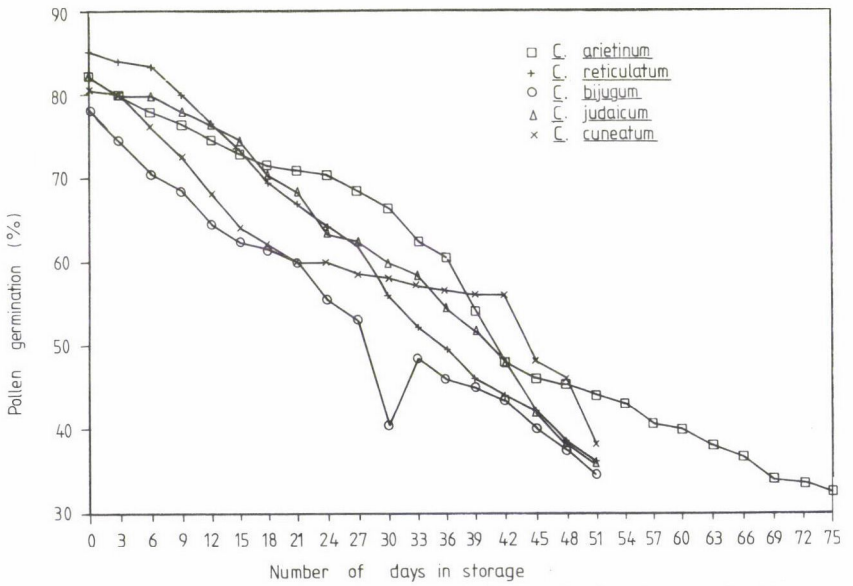

Fig. 2. Pollen germination percentage of different species of *Cicer* after keeping stored pollen on growth medium for 4 hours at 4 °C

Table 3

Percent pollen germination and pollen tube growth (μ) of different species of Cicer in various concentrations of growth regulators after 4 hours in the growth medium at 4 °C

Treatment	Conc. (μg/ml)	*C. arietinum* CV. C 235		*C. reticulatum*		*C. judaicum*		*C. bijugum*		*C. cuneatum*	
		Pollen germination (%)	Pollen tube length (μ)	Pollen germination (%)	Pollen tube length (μ)	Pollen germination (%)	Pollen tube length (μ)	Pollen germination (%)	Pollen tube length (μ)	Pollen germination (%)	Pollen tube length (μ)
1	2	3	4	5	6	7	8	9	10	11	12
Control	0	78.0	10.8	80.0	9.8	78.0	9.0	74.0	10.4	72.0	10.0
	5	82.0	11.0	80.0	10.2	78.0	9.4	72.0	11.0	74.0	10.2
Kn	10	88.0	11.2	86.0	10.8	86.0	10.2	84.0	11.6	82.0	10.4
	15	80.0	11.0	78.0	9.0	74.0	9.0	80.0	8.8	70.0	9.8
	5	80.0	10.4	82.0	10.2	74.0	9.8	70.0	9.6	72.0	8.6
ABA	10	82.0	10.8	87.0	10.8	82.0	9.8	80.0	10.4	86.0	9.8
	15	74.0	10.0	82.0	9.2	78.0	9.0	72.0	8.6	72.0	9.0
	5	76.0	10.8	78.0	10.0	75.0	8.4	70.0	10.0	72.0	9.0
IAA	10	78.0	10.8	83.0	10.5	84.0	8.8	82.0	11.2	84.0	9.8
	15	74.0	9.8	76.0	9.2	73.0	8.2	80.0	10.2	84.0	8.4
	5	82.0	10.8	80.0	10.8	72.0	8.2	74.0	10.4	74.0	10.4
GA	10	84.0	11.4	88.0	11.0	86.0	8.4	86.0	11.4	82.0	10.8
	15	76.0	9.4	78.0	10.0	74.0	6.8	76.0	8.6	74.0	9.6
	5	84.0	10.4	80.0	10.4	72.0	10.8	70.0	11.2	68.0	10.8
Kn 10+ABA	10	86.0	10.8	84.0	10.2	80.0	9.8	82.0	11.0	80.0	9.4
	15	80.0	9.4	80.0	9.0	72.0	8.2	68.0	8.2	68.0	8.2
	5	86.0	10.2	72.0	10.4	74.0	10.4	70.0	11.4	68.0	10.4
Kn 10+IAA	10	88.0	11.0	84.0	9.2	78.0	9.0	78.0	10.8	78.0	10.0
	15	80.0	7.2	80.0	8.0	68.0	8.0	72.0	6.2	62.0	8.8
	5	80.0	11.2	72.0	10.5	78.0	9.4	72.0	10.8	74.0	9.6
Kn 10+GA	10	84.0	9.8	84.0	9.4	80.0	8.0	86.0	11.2	82.0	8.0
	15	76.0	7.2	82.0	6.2	70.0	6.4	74.0	6.4	72.0	7.0
	5	78.0	12.0	68.0	9.8	70.0	10.2	72.0	11.4	70.0	9.2
ABA 10+IAA	10	80.0	11.4	82.0	9.2	78.0	8.2	74.0	10.0	78.0	7.8
	15	74.0	8.0	66.0	6.2	64.0	6.4	62.0	6.0	68.0	6.2
	5	80.0	11.2	78.0	10.0	72.0	11.0	76.0	10.2	75.0	9.8
ABA 10+GA	10	82.0	11.0	86.0	8.4	78.0	8.0	80.0	9.0	83.0	8.2
	15	76.0	9.8	68.0	6.4	60.0	6.2	62.0	8.0	59.0	6.4
	5	80.0	7.2	64.0	9.8	80.0	8.9	84.0	11.2	82.0	9.8
IAA 10+GA	10	78.0	8.4	82.0	11.4	68.0	10.6	86.0	10.5	80.0	10.8
	15	70.0	6.8	68.0	8.8	62.0	8.4	60.0	9.2	72.0	6.8
$\bar{X}$		79.87	10.04	78.65	9.50	75.42	8.80	75.10	9.84	74.74	9.13
S. E.		±0.76	±1.14	±1.14	±0.23	±1.07	±0.22	±1.25	±0.28	±1.18	±0.23
$LSD_{5\%}$		1.48	0.49	2.26	0.46	2.09	0.44	2.46	0.55	2.32	0.45

Experiments were conducted to study the effect of growth regulators on the percentile germination of pollen, as well as pollen tube growth, when supplied in the nutrient medium (Table 3). In general, pollen germination and tube length increased in a majority of the cases, with all growth regulators taken individually at 5 or 10 ppm. With an increase in their concentration both the parameters were negatively affected. The presence of Kinetin in the growth medium showed the best

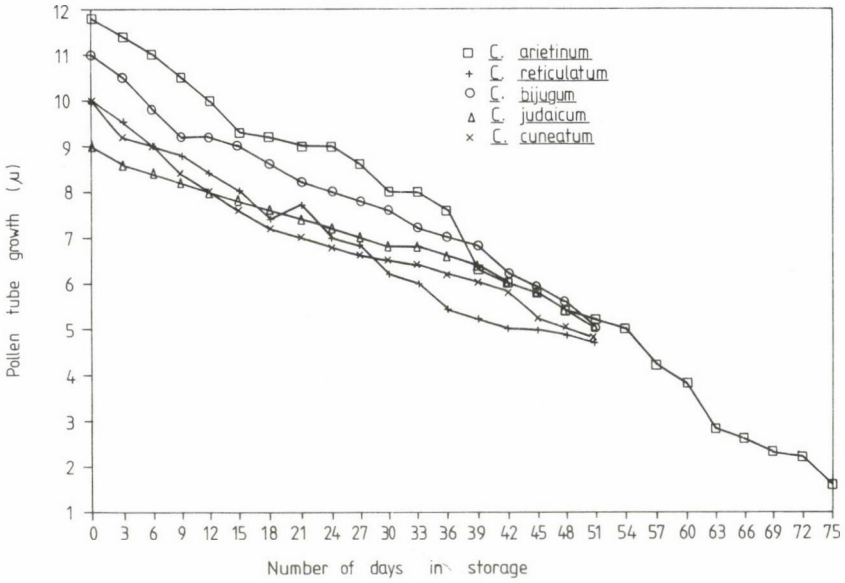

Fig. 3. Pollen tube growth (in microns) of different species of *Cicer* after keeping stored pollen on growth medium for 4 hours at 4 °C

results whereas abscisic acid seemed to be the least effective. The combination of Kinetin and abscisic acid had no positive effect on pollen tube growth, though it increased pollen germination at lower concentrations. The combination of abscisic acid and IAA showed a positive effect in the least number of cases.

Discussion

It was observed that pollen grains of *Cicer* remain viable (50%) for about 70 days depending upon the cultivars. Mercy et al. (1978) reported that even after storage for 42 days the pollen viability in *Cicer arietinum* was 43% while that of *C. soongaricum* was 46.3%. King (1965) and Chandra and Upadhyay (1978) suggested that the loss in pollen viability is due to a gradual degradation of different enzyme systems in the pollen, which can be checked by lowering the temperature and humidity during storage.

Sucrose, boric acid and calcium nitrate are the major constituents used by different investigators to facilitate pollen germination *in vitro*. The ideal sucrose concentration for the pollen germination was found to be 0.5 M. The highest germination, in the case of BG 315, indicated that its pollen was assimilating more sucrose and so its germination was faster. Though other varieties were showing a low percent germination of pollen, as compared to BG 315, their pollen tube growth was faster. This may be due to their better carbohydrate assimilation during tube growth than in the initial germination stage. Carbohydrate serves as a respiratory substance or provides precursors for tube wall synthesis (Thomas and Dnyasagar,

1975). At the higher and lower concentrations of sucrose there was lower pollen germination and tube length. It is possible that, at the higher concentration, too much osmotic pressure on the pollen wall may be inhibiting the germination, and at the lower concentration it may not be sufficient to provide enough nutrition to the pollen to germinate. When boric acid was provided in the nutrient medium, the best results were obtained in the case of 0.5 M sucrose and 100 ppm boric acid. The results indicated that the addition of boric acid further increased the percentage of pollen germination and tube length. Similar results were obtained by Mercy et al. (1974) in *Cicer* and Zhao et al. (1986) in *Brassica*.

In the initial days of storage, the pollen germination and tube growth in the nutrient medium was high, but with the increasing duration of storage there was considerable decrease. This effect was more profound in the wild species. A similar trend was reported by several workers in various crops (Chapliev, 1985; Gupta and Murty, 1985; Chaudhary and Shivanna, 1986; Borejko, 1987).

The effect of individual and combined effects of growth regulators on pollen germination and pollen tube growth in *Cicer* was also studied. The addition of Kinetin to the basal medium caused an increase in pollen germination at 5—10 ppm though the effect was not very consistent in all the species. Kinetin increased the final length of the pollen tubes with an increase in concentration up to 10 ppm, whereas with a further increase in its concentration it reduced pollen tube length. Ravindran and Chauhan (1986) reported that Kinetin treatments decreased germination and pollen tube elongation in *Solanum* with different concentrations from 1—25 ppm. According to them, an exogenous supply of Kinetin may have become toxic due to supraoptimal concentrations.

Pollen germination increased up to 10 ppm when ABA was added to the growth medium, whereas it decreased at higher concentrations. In general, the length of the pollen tube was less in the presence of ABA except at 10 ppm in some cases. The inhibitory effect of exogenous ABA may be due to its combined effect with an endogenous ABA-like inhibitor in pollen, or due to its known biochemical modifications (Walton, 1980).

When IAA was added to the growth medium, pollen germination increased at 10 ppm and pollen tube at 5 or 10 ppm. Higher concentrations were detrimental. According to Ravindran and Chauhan (1986), IAA from 1—25 ppm was harmful to both pollen germination and pollen tube growth in *Solanum*, and different species showed differential sensitivity.

In general, 100 ppm GA was helpful for pollen germination and pollen tube growth whereas higher concentrations were detrimental. Bose (1959) reported stimulation in *Pisum* whereas Dhingra and Varghese (1976) reported inhibitory effects of GA in *Lycopersicon*. GA-induced pollen tube elongation is considered to be due to its effect on cell expansion and orientation of newly synthesized microfibrils.

To determine whether these growth regulators could negate the inhibitory effect of each other, they were applied in combination. The results showed that Kinetin promoted pollen germination and pollen tube growth in combination with IAA and GA and also these growth regulators, in general, have a positive effect when taken together in the growth medium, on the two parameters studied. The

inhibitory effect of ABA was partially reversed by Kinetin. Such promotive effects due to the addition of IAA and GA into the basal medium have also been reported for plants and the work on these aspects has been comprehensively reviewed by Malik and Bhandal (1983).

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POLLEN TUBE STUDIES IN INTER-VARIETAL CROSSES OF CHICKPEA (*CICER ARIETINUM* L.)

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Four varieties of *Cicer arietinum* were taken to study pollen fertility, pollen germination and pollen tube behaviour in inter-varietal crosses, to determine if any abnormality in these is the cause of reduced crossability. There was not much difference in pollen sterility between different varieties. Pollen germination and pollen tube growth were more in selfings as compared to the crosses. Swelling of the pollen tube at the tip and other abnormalities were also recorded. A faster pollen tube growth and less number of abnormal pollen tubes could be directly correlated with more seed in the crosses.

Keywords: *Cicer arietinum*, emasculation, pollen, pollination

Introduction

India is one of the most important pulse growing countries in the world. Chickpea accounts for more than a third of the area and about 40% of the production of pulses in this country. However, the yield level of this crop has remained stationary over the last several decades. The natural genetic variability present within the cultivated chickpea has not yet been fully exploited and for some time to come it is most probable that the improvement of chickpea will depend on intraspecific hybridization.

Breeding advancement in the chickpea has been hampered by limited success in cross-pollination attempts (Turano et al., 1983). The high degree of self-pollination in chickpea is attributed to the fact that flowers are autogamous and that only certain papillate cells of the stigma are specialized to receive pollen when it is shed, prior to full flower bloom. During the white bud stage, such specialized cells produce a localized secretion which reacts positively to an esterase test. This test detects stigmatic surface proteins which are believed to be functionally important in the retention and hydration of pollen grains, and possibly indicates the site of the pollen-stigma compatibility reaction. These observations suggest that the low success rate of artificial hybridizations in chickpea may be related to the timing of the stigma receptivity and to the limited number of cells on the stigmatic surface which appear to be receptive to pollen. The present investigation was undertaken to study pollen fertility, pollen germination and pollen tube behaviour in inter-varietal crosses of *Cicer*, to determine if any abnormality in these is the cause of reduced crossability.

Akadémiai Kiadó, Budapest

Materials and methods

The experimental material used in the present study comprised four cultivated varieties of *Cicer arietinum* of which two are 'Desi' type, viz. C 235 and H 75—35, and two are 'Kabuli' type, viz. BG 315 and BG 422. All four varieties are resistant to *Ascochyta* blight.

The seeds were surface sterilised for ten minutes in 0.2% mercuric chloride. They were washed in distilled water and sown in the field. The following crosses were made:

1. C 235 × C 235
2. C 235 × H 75—35
3. C 235 × BG 315
4. C 235 × BG 422
5. H 75—35 × C 235
6. H 75—35 × H 75—35
7. H 75—35 × BG 315
8. H 75—35 × BG 422
9. BG 315 × C 235
10. BG 315 × H 75—35
11. BG 315 × BG 315
12. BG 315 × BG 422
13. BG 422 × C 235
14. BG 422 × H 75—35
15. BG 422 × BG 315
16. BG 422 × BG 422

Emasculation of flowers was done in the afternoon, taking care not to injure the delicate gynoecium or the flower, and avoid breaking the anthers. The gynoecium is not exposed as it is completely protected by keel petals, so bagging was not required after emasculation. Pollinations were done the next morning between 9—11 a.m. The styles along with the stigmas were detached from the top of the pollinated flowers with the help of forceps after 2, 24, 48, 72, 96 and 120 hours following pollination. These pistils were fixed in 1:3 aceto-alcohol for 24 hours and then preserved in 70% alcohol. They were macerated in 8 N NaOH for 4 hours, washed in distilled water and stained with cotton blue for one hour. The styles were washed with distilled water to remove the excess stain and kept for 20 minutes in 10% orthophosphoric acid, washed again and mounted in 50% glycerine.

Results

There was not much difference in pollen sterility between different varieties (Table 1). The pollen germination was found to be highest in selfings, as compared to the crosses (Table 2). There was a sequential increase in pollen germination with an increase in time after pollination, even up to 120 hours.

Table 1

Percent pollen sterility in different varieties of Cicer

Sl. No.	Varieties	Percent sterility
1.	*C. arietinum* cv. C 235	5.0 ± 0.81
2.	*C. arietinum* cv. H 75—35	4.0 ± 0.57
3.	*C. arietinum* cv. BG 315	6.0 ± 0.63
4.	*C. arietinum* cv. BG 422	7.0 ± 1.0

The emergence of pollen tubes on the stigma was seen right after two hours of pollination (Table 2). Pollen fall in the stigma of each individual was variable. In some cases 30—40 pollen grains were seen on the stigma while in others only 4—5 were observed. Pollen tube growth was faster after selfing, as compared to the crosses. Data on percentages of abnormal pollen tubes were also recorded (Table 2), which ranged from 6—20%. These abnormalities were in the form of swellings of the pollen tube at the tip. Many pistils showed one or several pollen tubes with swollen

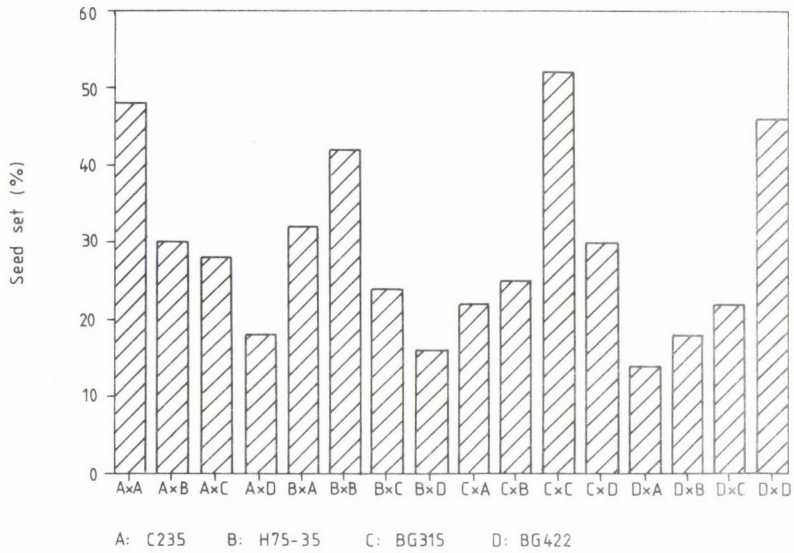

Fig. 1. Seed set (%) in intervarietal crosses of *Cicer arietinum*

Table 2

Percent pollen germination, pollen tube growth (μ) and percent abnormal pollen tubes (after 120 h), and percent pod set, abnormal pods and seed set in various crosses amongst different varieties/species of Cicer

Sl. No.	Crosses	Pollen germination (%)	Pollen tube growth (μ)	Abnormal tubes (%)	Pod set (%)	Deformed seeds (%)	Seed set (%)
1	2	3	4	5	6	7	8
1.	C 235 × C 235	66.0 ± 2.52	600 ± 15.16	9.5 ± 1.87	48.0 ± 6.57	0	48.0 ± 6.57
2.	C 235 × H 75—35	50.0 ± 3.22	480 ± 13.88	15.5 ± 2.65	38.0 ± 2.65	0	30.0 ± 5.57
3.	C 235 × BG 315	52.0 ± 3.22	385 ± 13.67	17.5 ± 2.31	28.0 ± 3.00	0	28.0 ± 3.00
4.	C 235 × BG 422	49.0 ± 5.57	390 ± 11.03	16.5 ± 2.00	18.0 ± 3.06	0	18.0 ± 3.06
5.	H 75—35 × C 235	52.0 ± 5.14	550 ± 19.58	14.5 ± 1.16	32.0 ± 3.00	0	32.0 ± 3.00
6.	H 75—35 × H 75—35	60.0 ± 2.52	580 ± 19.49	7.0 ± 2.62	42.0 ± 5.57	0	42.0 ± 5.57
7.	H 75—35 × BG 315	54.0 ± 3.06	480 ± 15.55	16.0 ± 3.61	24.0 ± 2.83	0	24.0 ± 2.83
8.	H 75—35 × BG 422	50.0 ± 4.59	460 ± 17.71	18.0 ± 2.08	16.0 ± 3.00	0	16.0 ± 3.00
9.	BG 315 × C 235	54.0 ± 1.53	520 ± 25.65	17.0 ± 2.00	22.0 ± 2.08	0	22.0 ± 2.08
10.	BG 315 × H 75—35	52.0 ± 4.17	530 ± 16.79	15.0 ± 2.65	25.0 ± 3.75	0	25.0 ± 3.75
11.	BG 315 × BG 315	64.0 ± 4.73	650 ± 14.44	8.5 ± 3.00	52.0 ± 5.57	0	52.0 ± 5.57
12.	BG 315 × BG 422	52.0 ± 2.65	510 ± 14.02	16.0 ± 2.00	30.0 ± 4.05	0	30.0 ± 4.05
13.	BG 422 × C 235	50.0 ± 1.92	580 ± 31.38	19.0 ± 2.08	14.0 ± 2.52	0	14.0 ± 2.52
14.	BG 422 × H 75—35	48.0 ± 2.52	580 ± 6.12	20.0 ± 3.22	18.0 ± 3.59	0	18.0 ± 3.59
15.	BG 422 × BG 315	49.0 ± 2.08	520 ± 16.31	17.5 ± 1.53	22.0 ± 4.51	0	22.0 ± 4.51
16.	BG 422 × BG 422	62.0 ± 2.00	680 ± 23.89	6.0 ± 2.00	46.0 ± 3.52	0	46.0 ± 3.52

tips. Some pollen tubes showed twisting and swelling on the stigma, whereas others showed more than one tube coming out from the same germpore.

Maximum seed set was obtained in selfings (Fig. 1). In BG 315 × BG 315 a maximum seed set (52%) was recorded while in BG 422 × C 235, the least seed set (14%) was obtained.

Discussion

Pollen sterility may be due to poor growth of the plant, chlorophyll deficiency, chromosomal aberrations, etc. Microtomic studies revealed that tapetum plays a definite role in the development of microspores (Chauhan, 1976). Behl et al. (1974) found late degeneration of tapetum leading to pollen abortion in triticale-wheat hybrids.

Considerable differences were observed in the germination of self or the cross pollen grains with the former being much higher. Turano et al. (1983) attributed the high degree of self pollination in chickpea to the fact that flowers are autogamous and that only certain papillate cells of the stigma are specialized to receive pollen when it is shed prior to flower bloom. They suggested that a low success rate of artificial hybridization in chickpea could be related to the timing of stigma receptivity and to the limited number of cells on the stigmatic surface which appear to be receptive to pollen. Singh and Khanna (1988) reported a great difference in pollen germination between readily crossable and poorly crossable types in wheat, triticale and rye crosses.

The germinated pollen grains usually produced thick pollen tubes near the stigmatic surface. However, as they travelled down the style, the pollen tubes became slender and intensity of the staining decreased. By comparing the pollen tube development with the time needed for the first tube to reach the embryo sac, Hoshikawa (1960) observed in wheat that only the early germinating grains and the fast-growing pollen tubes are of interest with regards to fertilization.

A study was made on the development of pollen tubes in the different crosses to determine whether the hindrance to normal growth of pollen tubes in some cases was due to the abnormal development of pollen tubes. These abnormalities were 6—10% on selfing and 14—20% in the crosses. Singh and Khanna (1987) also reported these abnormalities in wheat-rye and triticale-wheat crosses. Jaitly (1989) reported that pollen tube abnormalities in intervarietal crosses in rice decreased seed set to some extent.

Looking at the above results it is clear that seed set is more on selfing as compared to the crosses. Moreover, 'Desi' × 'Desi' or 'Kabuli' × 'Kabuli' resulted in more seed set than 'Desi' × 'Kabuli'. A faster pollen tube growth and less number of abnormal pollen tubes could be directly correlated with more seed in the crosses.

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OCCURRENCE AND PHYSIOLOGICAL ROLE OF BENZOXAZİNONES AND THEIR DERIVATIVES. I. CYTOKININ ACTIVITY OF 6-METHOXY-BENZOXAZOLINONE

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With alcoholic extracts of maize internodes applied in ethyl acetate–formic acid–water (60 : 5 : 35) solvent, three cytokinin activity zones appeared on the paper chromatogram. In the active zone of about $Rf = 0.2$ a minor quantity of zeatin and a larger quantity of zeatinriboside were identified. In the zone around $Rf = 0.6$ substance(s) with so far unknown cytokinin activity was (were) found. The cytokinin activity compound of the zone behind the front line was prepared in crystalline form too. By mass spectrography the compound was identified as 6-methoxy-benzoxazolinone.

In *Amaranthus* betacyanine test the cytokinin activity of this compound increases between 0.1 and 1.0 mg/mL, while at higher concentrations the activity decreases.

Keywords: cyclic hydroxamic acids, cytokinin-active compounds of maize, 6-methoxy-benzoxazolinone, zeatin, zeatinriboside

Introduction*

Data on the occurrence and role of cyclic hydroxamic acids were first published by Virtanen et al. (1956). From maize and wheat an antifungal compound proved 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazine-3(4H)-one was identified. From rye 2,4-dihydroxy-2H-1,4-benzoxazin-3(4H)-one was also isolated (Hietala and Virtanen, 1960). In plant tissues these compounds are to be found in glycoside bond. In the course of tissue destruction the glycosidases release the aglucones. The cyclic hydroxamic acids are compounds readily decomposing, in aqueous media under warming they transform into benzoxazolinone or 6-methoxy-benzoxazolinone, while formic acid is released (Virtanen and Hietala, 1959).

* Abbreviations:
Cyclic hydroxamic acids:
DIBOA: 2,4-dihydroxy-2H-1,4-benzoxazine-3/4H/one
DIMBOA: 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazine-3/4H/one
DIM_2BOA: 2,4-dihydroxy-7,8-dimethoxy-2H-1,4-benzoxazine-3/4H/one
Benzoxazolinones:
BOA: benzoxazolinone
MBOA: 6-methoxy-benzoxazolinone
DMBOA: 6,7-dimethoxy-2-benzoxazolinone

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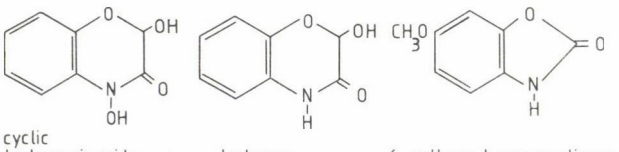
Benzoxazinones were isolated from roots of Coix lacryma-jobi too (Koyama, 1955). From aqueous extract of maize seedlings, after ethyl acetate extraction Woodward et al. (1979) identified three hydroxamic acids (DIBOA, DIMBOA, DIM_2BOA), three related lactames (HBOA, HMBOA, 7-OH MBOA) and 6-methoxy-benzoxazolinone (MBOA). The benzoxazinones are heterocyclic compounds, in the hexamerous ring oxygen and nitrogen are the heteroatoms. In the cyclic hydroxamic acids hydroxyl, in the lactames hydrogen joins to the nitrogen. The skeleton of the cyclic hydroxamic acids (benzoxazinones), of the related lactames, and the structure of 6-methoxy-benzoxazolinone are shown by the following formulae:



The aromatic ring of the benzoxazolinones is synthetized via shicimic acid pathway the immediate precursor is anthranilic acid, while the oxazine ring is formed from ribose phosphate (Tipton et al., 1973). The biosynthesis of the compound in its first phase agrees with the formation of tryptophane. Of the three cyclic hydroxamic acids of maize DIMBOA occurs in nearly 1%, while the other two in 0.1%, in terms of dry weight (Klun et al., 1970). Tang et al. (1975) when examining the occurrence of benzoxazolinones in grasses by gas chromatography, found them in considerable quantities (0.12—2.61 mg/fresh weight) in maize-, wheat-, rye- and Coix lacryma-jobi tissues. On the other hand, tissues of rice, barley, oat and millet did not contain demonstrable amounts of benzoxazolinones.

The cyclic hydroxamic acids, and their derivatives once were in the centre of interest. The increased interest can be explained by the fact that these compounds were thought to have something to do with resistance to fungi, bacteria, insects, and herbicides containing 2-chloro-s-trizine as active agent. According to Ingham (1972) under physiological conditions benzoxazolinones are not formed in plant tissues, so their role in resistance is questionable. Since, however, the precursors (the appropriate cyclic hydroxamic acids and their glycosides) also possess antifungal properties, it is possible that they have a part in resistance. For example, in wheat varieties resistant to *Puccinia graminis tritici* the concentration of glycoside in the cyclic hydroxamic acid is higher than that in the susceptible varieties (Elnaghy and Linko, 1962; Elnaghy and Shaw, 1966). Their physiological role from the aspect of plants is hardly — if at all — known. Concerning the physiological role of benzoxazolinones a peculiar idea has arisen. The maize coleoptiles contain a substance of low molecular weight, which modifies the linkage of auxin. The substance called supernatant factor decreases the affinity of the naphtyl acetic acid to the auxin receptors localized in the membrane. Venis and Watson (1978) identified this factor with the mixture of 6-methoxy-2-benzoxazolinone (MBOA) and 6,7–dimethoxy-2-benzoxazolinone (DMBOA). They found that DMBOA was about 50-times more

active than MBOA. These compounds inhibit the auxin induced growth of oat coleoptiles too, and effect is in correlation with the blocking of the places of auxin linkage. Since the precursors of these compounds occur only in certain grass species, they cannot be regarded as general modulators of the auxin effect. In the course of analysing the cytokinins of maize tissues we detected cytokinin-active compounds which could not be identified with any of the so far known cytokinins. One of them proved to be 6-methoxy-benzoxazolinone. In the present paper we give account of these investigations.

Materials and methods

For the experiments shoots of five-day-old two-leaf maize plants grown in a growth chamber, and still elongating (non-fibrillized) internodes from field experiments, both of the two-line hybrid Pioneer 3950 MSc were used. The seedlings were raised in 16-hour illumination at 25 °C, and in 8-hour dark period at 20 °C, resp., in perlite moisted with Knop's culture solution. The tissues were placed in 95% ethanol of triple quantity, then cooled down to −20 °C, and homogenized for 1 minute in Biomix knife-homogenizator. The cold homogenizate was centrifuged. The sediment was taken up by 70% ethanol of triple quantity, and extracted by continually mixing it overnight at 4 °C, then centrifuged. The two alcoholic extracts were combined, and concentrated in vacuum to small volume at a temperature not exceeding 40 °C. A considerable proportion of proteins, lipids and sugars was precipitated with a three fold amount of ethanol. The sediment precipitated cold was removed in centrifuge. The alcoholic solution was concentrated in vacuum, taken up with water of a quantity corresponding to 1/5 of the fresh mass, then shaken three times with 1-butanol of the same volume at pH 8.4. The aqueous phase was discarded. The butanolic phase mostly was rinsed with water, then concentrated in vacuum, and the residue taken up with 70% ethanol. The alcoholic samples were stored at −20 °C until processed.

DIMBOA was extracted from seedlings using the method of Woodward et al. (1978). From the aqueous extracts the DIMBOA was extracted with diethyl ether. Purification was carried out on Sephadex LH-20 column with 35% ethanol. The volume of column was 180 ml, the through-flow velocity 25 ml/h, the volume of fractions 12.5 ml. The fractions were tested with $FeCl_3$ solution using drop analysis. Namely, the hydroxamic acids with trivalent iron ions form coloured complexes (Hamilton, 1964). Fractions giving blue colour were combined and concentrated in vacuum. The slightly yellow crystalline residue was dissolved in ethanol. For paper chromatography Whatman 3 MM paper, a mixture of i-propanol–NH_4OH–water (10 : 1 : 1) and ethyl acetate–formic acid–water (60 : 5 : 35), ethyl acetate–n-propanol–water (4 : 1 : 2) as solvent, while in the case of thin layer chromatography Kieselgel 60 F_{254} plates and a 9 : 1 ratio mixture of chloroform and methanol as solvent were used. In order to remove the solvent residues of paper chromatograms the papers were dried with cold air for 24 hours. In the meantime, they were twice sprayed with ion-free water and dried with hot air (45 °C) for 10 minutes on each occasion. As it turned out later, the treatment played a decisive role in the chemical processes taking place on the paper. The cytokinin activity was measured with *Amaranthus* betacyanine test (Biddington and Thomas, 1973). The extinction of the betacyanine solution dissolved after repeated freezing and melting from shoots of three-day-old *Amaranthus caudatus* seedlings deprived of root was measured at 542 nm.

The paper equal in size with the test paper, originating from the chromatogram below the start line served as control. The extinction of this was subtracted from the extinction shown by the test solution. The thousandfold value of the difference thus obtained was represented in diagram. The mass spectrometric examinations were carried out with direct sample intake, using a VG-7032 type mass spectrometer (VG Analytical Ltd., England), in electronic collision way of ionization (EI) at the following parameters: ionization energy = 70 eV; temperature of ion source = 200 °C; break-down = 1000.

Results and discussion

Cytokinin-active compounds of maize internodes

As a first step we tried to find such a method of purification and separation that would make it possible to process the large number of samples in a relatively simple way, eliminate the disturbing materials and separate the cytokinin-active compounds with the best possible result. In our preliminary investigations we found that with paper chromatography none of the classical solvents resulted in satisfactory separation, because most cytokinin-active compounds had similar *Rf*-values.

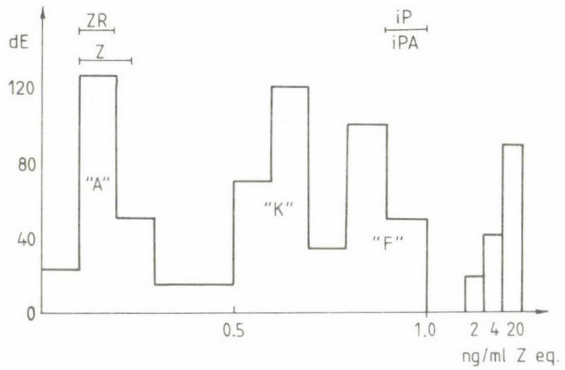


Fig. 1. Distribution of cytokinin-active compounds extracted from the two lower internodes of four weeks old maize plants in a chromatogram developed with ethyl acetate–formic acid–water (60 : 5 : 35) solvent. *Amaranthus* betacyanine test, 4 g fresh weight/mL thyrosin phosphate buffer. Z, ZR, iP and iPA: position of standards in the chromatogram, on the horizontal axis activity corresponding to 2, 4, 20 ng/mL zeatin

In the most widely used isopropanol–ammonia–water (10 : 1 : 1) solvent the cytokinin-active compounds are to be found in a relatively well-separated band ($Rf = 0.65$—0.85), while the disturbing materials appear at lower *Rf*-values. So this solvent is highly suitable for the preliminary purification of the crude extracts.

According to our preliminary studies for the paper chromatographic separation of preparations made from alcoholic extracts of maize internodes the upper phase of the mixture of ethyl acetate–formic acid–water (60 : 5 : 35) is the most suitable solvent. The separation is quick, and the most active zones were observed here. As seen in Fig. 1, with the extracts of young maize internodes applied in this solvent on Whatman 3MM paper, three active regions can be distinguished: the lower region (hereinafter: A-fraction) shows maximum activity at 0.2 *Rf*-value, while in the medium zone (K-fraction) the activity appears at about 0.6 *Rf*-value. Close to the front line (F-fraction) a cytokinin-active compound best solved in ethyl acetate can be detected.

After that we attempted the identification of the cytokinin-active compounds of the three fractions. Of the four most generally occurring natural cytokinins the

zeatin and zeatinriboside show 0.2 *Rf*-value in the ethyl acetate–formic acid–water solvent. The isopentenyl-adenine (iP) and the isopentenyl-adenozine (iPA) are located close to the front line, though somewhat below the F-fraction. The three fractions separated with the ethyl acetate–formic acid–water solvent were extracted from the paper with ethanol, then run in 30 mmol borate buffer (pH = 8.4).

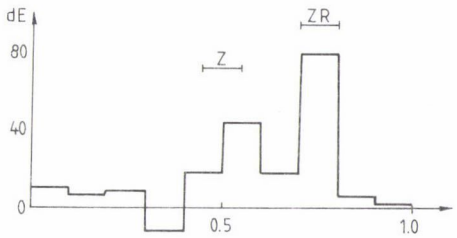


Fig. 2. Distribution of compounds extracted from the lower region (*Rf* = 0.1—0.3) of a chromatogram developed with ethyl acetate–formic acid–water (60 : 5 : 35) solvent in 30 mmol borate buffer (pH = 8.4)

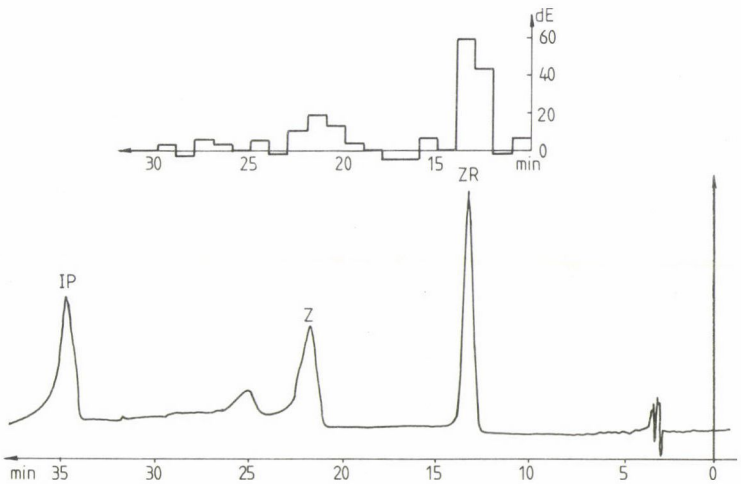


Fig. 3. Analysis of cytokinin-active compounds eluated from the A-zone of a chromatogram developed with ethyl acetate–formic acid–water solvent, in an intensive liquid chromatography system. The lower curve of the figure shows the retention time of the zeatin ribosid (ZR) and zeatin (Z) standards as detected at 254 nm. The diagram represents the cytokinin activity of 1 mL fractions taken at intervals of one minute in Amaranthus betacyanine test. Column: 25 cm long Chromsil C_{18}, with a charge of 10 μm grain size. Elution with a mixture of methanol and 0.02 mol ammonium acetate buffer (pH = 3.5). Methanol concentration 20% in the first two minutes, increasing by 4% every minute for 5 minutes. The 50% concentration of methanol kept for 5 minutes, then increased to 70% during further 5 minutes. The flow rate was: 1.0 mL/min

As seen in Fig. 2 the zeatin and zeatinriboside separate well from one another in this solvent, and the two active zones of the extract show the same Rf-values as the cytokinins. Similar conclusions were drawn from examinations by liquid chromatography (Fig. 3). Accordingly, in the A-region of the chromatogram developed

with ethyl acetate–formic acid–water solvent zeatin and zeatinriboside can be found. The proportion of the zeatinriboside is considerably larger. Here we do not deal with the identification of the K-fraction.

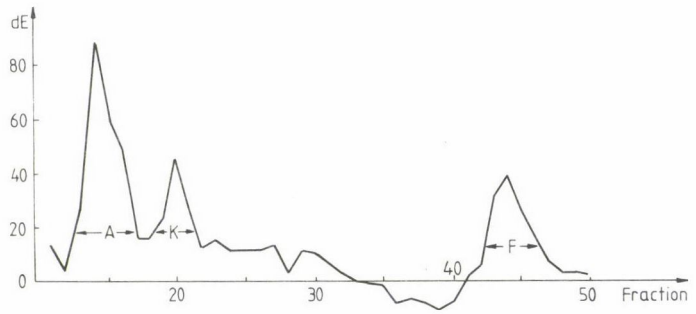


Fig. 4. Separation of purified extract of differentiating maize internodes on Sephadex LH-20 column. Cubic capacity of column: 180 ml, elution with 35% ethanol. Velocity of through-flow: 20 mL/h, volume of fractions: 10 ml. The cytokinin activity was determined in *Amaranthus* betacyanine test

Isolation and identification of 6-methoxy-benzoxazoline-2-one

Since by paper chromatography the F-fraction could not be identified with any known cytokinins, for the chemical analysis we tried to isolate a larger amount. For this purpose the butanol fractions were purified on Whatman 3 MM paper in isopropanol–ammonia–water (10 : 1 : 1) solvent, the $Rf = 0.65$—0.85 region was extracted with ethanol, then separated on Sephadex LH-20 column with ethanol. As seen in Fig. 4 the A- and K-fractions do not separate, while with a higher fraction number the F-fraction definitely separates. The active fractions were identified in ethyl acetate–formic acid–water solvent. After that for the preparation of the F-fraction the following purification processes were used:

— The basic butanol extract was chromatographed in isopropanol–ammonia–water solvent on Whatman 3 MM paper, the active region extracted with ethanol.
— Separation took place on Sephadex LH-20 column. The active fractions were combined and concentrated.
— Purification on preparative silicagel plate. The biologically active zone was scraped from the plate, the material was extracted with ethanol. So we obtained a crystalline needle distillation residue.

The purified F-fraction was then chromatographed by way of intensive liquid chromatography on reverse phase column with methanol–water eluent. As seen in Fig. 5 the F-fraction shows a polarity similar to zeatinriboside.

The rather clear F-fraction was then submitted to chemical analysis. The EI(+) mass spectrum of the sample can be seen in Fig. 6. The base peak of the mass spectrum, which is at the same time the peak of the molecule ion of the substance analysed ($M^{\cdot+}$), appears at $m/z = 165$. With the fragmentation properties of the molecule ion ($M^{\cdot+}$) interpreted, the compound is 6-methoxy-benzoxazoline-2-one.

The effective concentration of 6-methoxy-benzoxazolinone

Even during the preparation work we found that between the absorption curve of the eluate running off the column and the cytokinin activity of the fractions there was a correlation characteristic of a saturation curve (Fig. 7). The eluate showed absorption maximum at fraction 27, while fractions 26 and 29 hardly differed in cytokinin activity. Subsequently, we examined the cytokinin activity in two preparations of different origin and purity, stored for different times at −20 °C

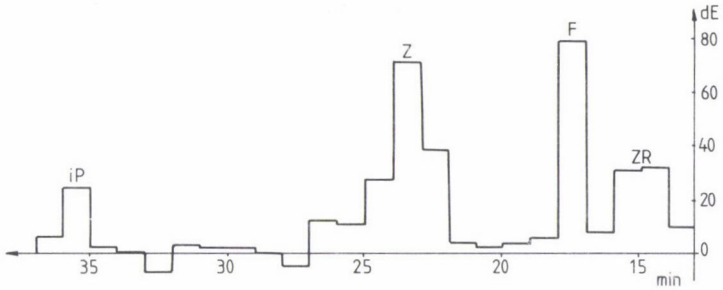


Fig. 5. Retention of zeatin, zeatinriboside, isopentenyl adenine, as well as of the chromatographically purified "F"-fraction on Chromsil C_{18} column, and their cytokinin activity in *Amaranthus* biotest. Details of the analysis in Fig. 3

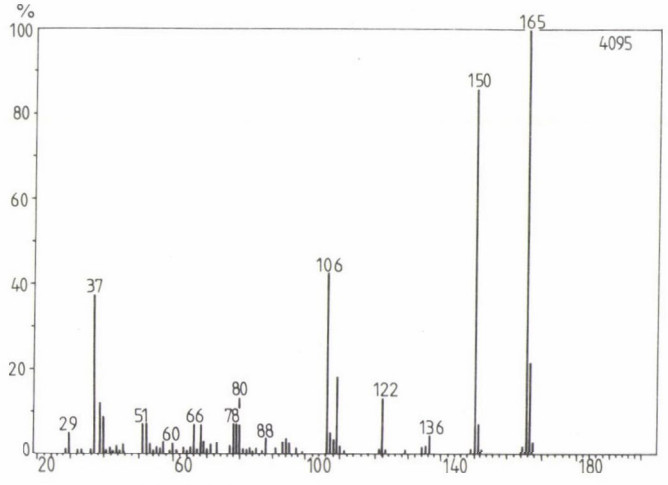


Fig. 6. Mass spectrum of the F-fraction

(Fig. 8). Although the experiment data for the two preparations show considerable differences, due to the differences between them in degree of purity, age, and so in activity too, it still can be established that between the concentration and cytokinin activity of MBOA there is a correlation that can be described with a saturation curve. At a lower level of concentration (0.1 and 0.6 mg MBOA per mm test

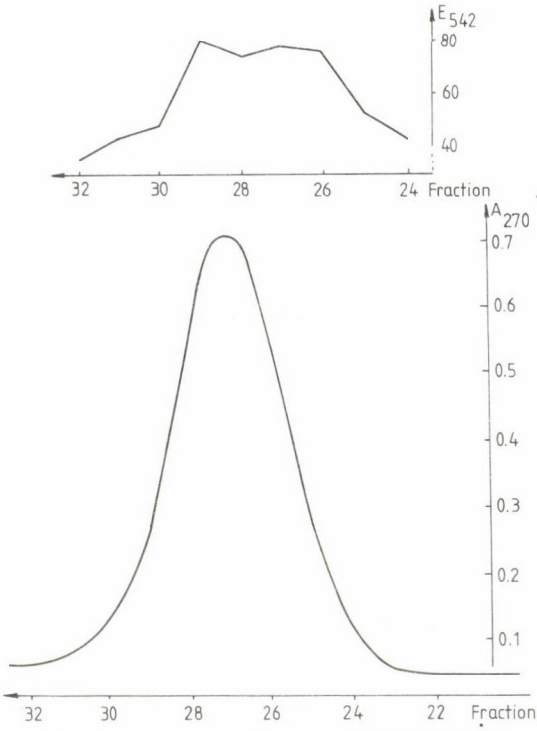


Fig. 7. Absorption curve of 6-methoxy-benzoxazolinone purified with M/75 phosphate buffer (pH = 6.4) on polyvinyl-pirralidon column, and cytokinin activity of fractions gathered every 30 minutes. Cubic capacity of column: 95 mL, speed of through-flow: 29.5 mL/h; detection at 270 nm

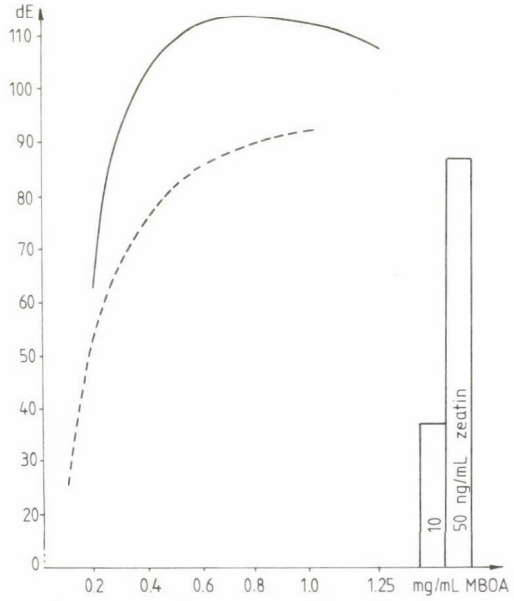


Fig. 8. Correlation between the concentration and cytokinin activity of two different MBOA preparations

solution) the betacyanine production increases, then the specific cytokinin activity becomes more and more moderate; at higher MBOA concentrations the cytokinin activity not only stops increasing but it even decreases. On the basis of comparative analyses with zeatin it can be established that the maximum cytokinin activity shown by 1 mg/mL MBOA corresponds to about 50 ng/mL zeatin activity, that is, the zeatin is 10^4–10^5-times more active than the MBOA.

In terms of fresh weight, from maize seedlings some 20 mg MBOA per 100 g could be isolated. With the losses of preparation taken into consideration, this concentration would be sufficient for a rather high cytokinin activity. It is not likely, however, that in living organisms the precursors of MBOA are able to transform into cytokinin-active compounds. Therefore, the cytokinin activity of 6-methoxy-benzoxazolinone is of mere theoretical and methodical importance, its *in vivo* role is doubtful. In the case of grass species in which cyclic hydroxamic acids occur, the disturbing effect of their decomposition products must be reckoned with.

Acknowledgements

The authors are indebted to Prof. Sándor Makleit, Head of the Department of Organic Chemistry, Kossuth Lajos University, Debrecen, for his valuable help in identifying the 6-methoxy-benzoxazolinone, and to Mrs. Erzsébet Faragó for her devoted laboratory work.

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OCCURRENCE AND PHYSIOLOGICAL ROLE OF BENZOXAZINONES AND THEIR DERIVATIVES. II. DECOMPOSITION OF 7-METHOXY-BENZOXAZINONE AND CHANGE IN ITS PHYSIOLOGICAL ACTIVITY

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The 7-methoxy-benzoxazinone isolated from maize seedlings is toxic to *Amaranthus caudatus* seedlings. When heated in aqueous media, or in the presence of ammonia even at room temperature the compound decomposes into several products including 6-methoxy-benzoxazolinone, which in *Amaranthus* betacyanin test shows cytokinin activity. The transformation takes place on chromatographic paper or silicagel plate, too. In the presence of 0.03 mol/L NH_4OH at 60 °C the transformation is practically complete in 5 minutes and no other detectable decomposition products occur.

Keywords: cytokinin-active compounds of maize, phytotoxicity, 6-methoxy-benzoxazolinone, decomposition of 7-methoxy-benzoxazinone

Introduction

In the course of processing alcoholic extracts of maize tissues we identified a cytokinin-active compound which proved to be 6-methoxy-benzoxazolinone (Pethő and Dinya, 1991). This compound has long since been known (Virtanen and Hietala, 1959) to be produced from the corresponding benzoxazin, when the latter is heated in aqueous media, — while formic acid is released. The half-time of the 7-methoxy-benzoxazinone in aqueous media close to neutral (pH = 6.75) at 28 °C is only 5.3 hour (Woodward et al., 1978). Accordingly, the 6-methoxy-benzoxazolinone is an artificial preparation product which cannot occur in intact maize tissues. Since the physiological role of the benzoxazins has not been sufficiently cleared, it is desirable to study their stability, as the physiological role of a readily decomposing compound cannot be examined without knowing the conditions of stability.

In the maize tissues two methoxy derivatives of the benzoxazin also occur (Woodward et al., 1979). The native form of these compounds is to be found in glycoside bond. In the course of homogenization at room temperature the glycosidases are not inactivized during the usual alcoholic extraction and they break up the glycosides.

Of three benzoxazins (DIBOA, DIMBOA and DIM_2BOA) the 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA, or in short: 7-methoxy-benzoxazinone) occurs in the largest amount in maize tissues. Our attention was concentrated on this compound. Of the occurrence and possible physiological role

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of the benzoxazins (cyclic hydroxamic acids) we gave account in detail in an earlier paper (Pethő and Dinya, 1992). The genetic relation between the compounds concerned is shown in Fig. 1.

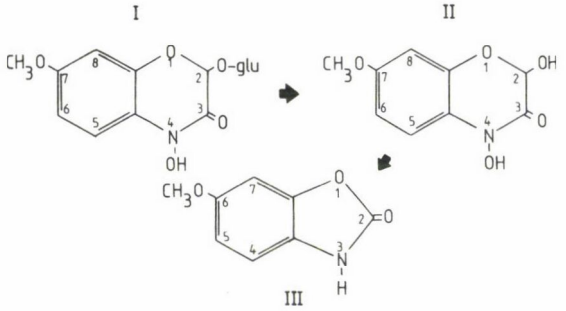

Fig. 1. Structure and genetic relation of DIMBOA-glucoside (I), DIMBOA (II) and MBOA (III)

Materials and methods

Etiolated seedlings of Pioneer 3950 MSc two-line hybrid grown for five days between wet filter paper in dark, at a 25 °C thermostat were homogenized with a double volume of water, then kept at room temperature for 50 minutes in order to make the glycosidases release the DIMBOA from the glycoside bond. After rapid heating and cooling the precipitated proteins were separated in centrifuge. From the supernatant the DIMBOA was extracted with diethyl ether (Woodward et al., 1978). The crude extract was purified on Sephadex LH—20 column with 35% ethanol. The $FeCl_3$-positive fractions were combined and concentrated dry in vacuum. The residue was dissolved in 70% ethanol.

For paper chromatography Whatman 3 MM paper, a mixture of i-propanol–ammonia–water (10 : 1 : 1) and ethyl acetate–n-propanol–water (4 : 1 : 1) as solvent, for thin layer chromatography Kieselgel 60 plates and a mixture of chloroform and methanol (9 : 1) as solvent were used.

The cytokinin activity was determined by the betacynin test of Biddington and Thomas (1973). From 7-methoxy-benzoxazinone preparation stored in 70% ethanol at −20 °C adequately diluted aqueous solution corresponding to 2 g fresh tissue per mL was made. For ammonia treatment NH_4OH of a quantity corresponding to 0.03 mol/L was added to the solution. The solutions were incubated at 25 °C for different lengths of time. In the case of heat treatment the solution was placed in water-bath of 50 or 60 °C for different lengths of time. After heat treatment the solution was stored in ice-bath. The solutions were analysed by liquid chromatography in LABOR MIM-type Liquochrom apparatus, on a 25 cm long Chromsil C_{18} reverse-phase column.

On the column a 20 microlitre sample was applied. Solvent: 50% methanol containing 0.02 mol NH_4-acetate (pH = 3.5). The flow rate: 1 mL/min, detection at 290 nm, final deviation 0.5 absorbance. The methods were described in detail in an earlier paper (Pethő and Dinya, 1992), some of them will be shown in the course of presenting the results.

Results and discussion

Origin of 6-methoxy-benzoxazolinone

Studying the cytokinin-active compounds of alcoholic extracts from maize tissues we found phytotoxic or cytokinin-active zones — depending on the solvent — in the extracts purified by paper chromatography. When extracts prepared from

shoots of five-day-old maize seedlings were applied on Whatman 3 MM paper in ethyl acetate–n-propanol–water (4 : 1 : 2) solvent, cytokinin activity was hardly detected in the different zones with the *Amaranthus* test. When, on the other hand, the same extract was chromatographed in isopropanol–ammonia–water (10 : 1 : 1) solvent, in the Rf = 0.7 region very intensive cytokinin activity was observed (Fig. 2).

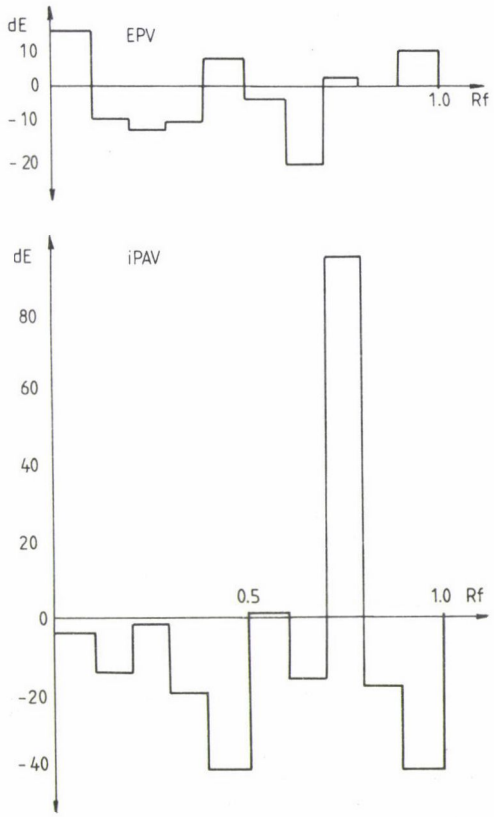

Fig. 2. Paper chromatographic separation of a preparation obtained from shoots of five-day-old maize seedlings by extraction with ethanol, then purified with butanol, using ethyl acetate–propanol–water (4 : 1 : 2) and isopropanol–ammonia–water (10 : 1 : 1) as solvent. Amount of material: a quantity of extract equal to 0.5 g fresh weight per mL test solution

On the basis of literary data we supposed that the 6-methoxy-benzoxazolinone originated from 7-methoxy-benzoxazinone (DIMBOA) through decomposition while chromatographed in basic medium (ammonia-containing solvent). This hypothesis was supported by our observation that when the ethyl acetate chromatogram was dried with hot air cytokinin activity, while in the case of cold air drying phytotoxicity was found in the same region.

To prove our hypothesis correct we applied purified DIMBOA preparations on silicagel plates. The developed chromatogram was submitted to various treatments, then the different zones were examined in biological test for cytokinin activity. As

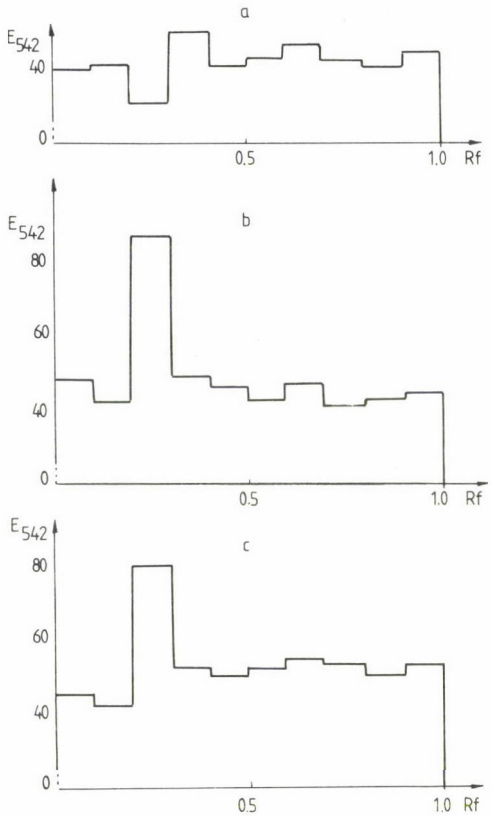

Fig. 3. Transformation of DIMBOA into MBOA on silicagel plates. The purified DIMBOA preparation was chromatographed on Kieselgel 60 F_{254} plate in a mixture of chloroform–methanol (9 : 1); *a:* untreated plate; *b:* plates kept in an air space with ammonia for 24 hours, or *c:* in hot steam for 10 minutes, then in a space saturated with steam for 24 hours

seen in Fig. 3, on plates kept in ammonia vapour, or for 10 minutes in hot steam, then for 24 hours in a space saturated with steam, the toxic material observed in the $Rf = 0.3$ region was transformed; in this region cytokinin activity was observed on the treated plates. With these experiments we rendered it probable that while chromatographed in solvents containing ammonia, or dried with hot air, on paper the DIMBOA was transformed into MBOA. Then the purified DIMBOA solution was heat treated in water-bath for 50 minutes, and chromatographed on silicagel plates. As seen in Fig. 4 the *Rf*-value of the toxic DIMBOA in the case of chloroform–methanol (9 : 1) solvent used is 0.3, while that of the cytokinin-active compound formed after heat treatment is 0.5.

Effect of heat treatment and ammonium ions on the decomposition of 7-methoxy-benzoxazinone

Since in the course of the chromatographic procedures we found that both the temperature and the ammonium ions influenced the decomposition of DIMBOA, we studied the stability of the compound by liquid chromatography in order to obtain more exact data. On Chromsil C_{18} column, with 50% methanol used as solvent, the DIMBOA has a 5.8-minute retention time. In the presence of ammonia its retention changes, the peak appears between 6.4 and 6.8 minutes (Fig. 5).

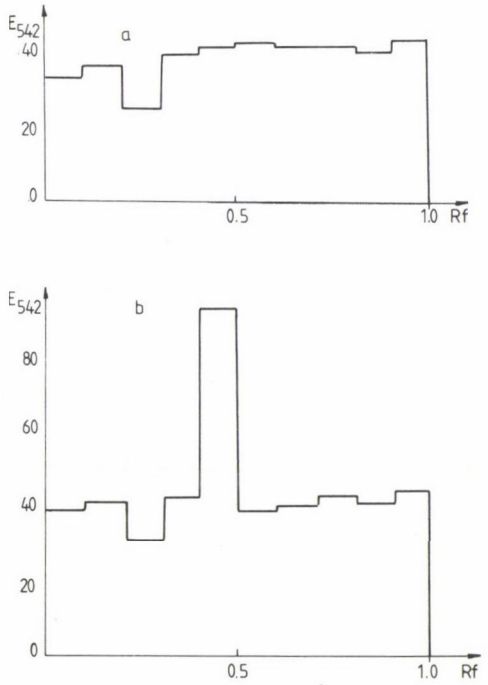

Fig. 4. Separation of untreated *(a)* and 50-minute heat treated *(b)* purified DIMBOA on Kieselgel 60 plates in a mixture of chloroform–methanol (9 : 1) then examined for cytokinin activity in *Amaranthus* bioassay

With the solution incubated at room temperature for 5 minutes then chromatographed, the shoulder of the curve appears at about 7.5 minutes and becomes more and more definite with the prolongation of the incubation time. In the chromatograms of samples kept for 40 minutes at room temperature, then heat treated for 3 minutes at 50 °C, the shoulder of the curve appears as a further peak. At the same time well definable other decomposition products appear with 3.4- and 5.2-minute retention time. Parallel to this the area below the curve attributable to DIMBOA considerably decreases in size.

After that to preparations containing DIMBOA corresponding to 5 g fresh weight per mL, ammonium hydroxide equal in quantity to 0.03 mol/L was added. During the experiment the preparations were kept in ice-bath (control), or incubated for 5 minutes in water-bath of 60 °C. The two preparations were liquid chromatographically analysed. A comparison of the two HPLC chromatograms

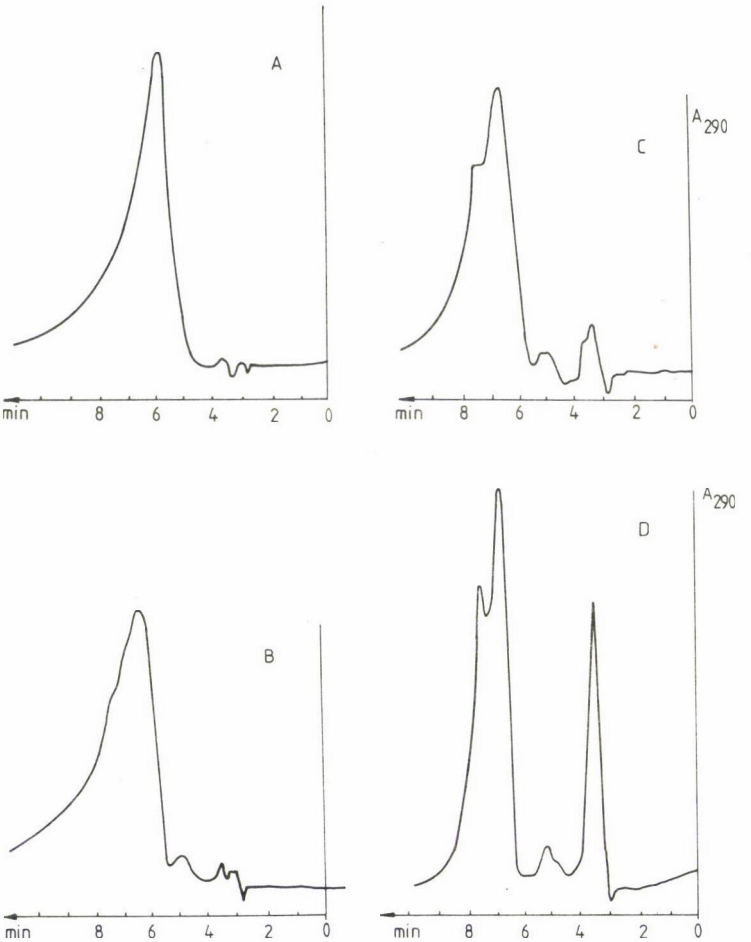

Fig. 5. Effect of ammonia and heat treatment on the decomposition of DIMBOA. *A:* control, NH_4OH is replaced by an identical volume of water; *B:* DIMBOA solution completed with ammonia, incubated for 5 minutes at room temperature; *C:* the same for 30 minutes; *D:* incubated for 40 minutes at room temperature, after 30 minutes heated to 50 °C for 3 minutes

(Fig. 6) makes it clear that in the presence of NH_4OH at 60 °C the transformation of DIMBOA into MBOA takes place in 5 minutes. The relatively short time of heat treatment did not result in any considerable decomposition, in the appearance of large quantities of other decomposition products.

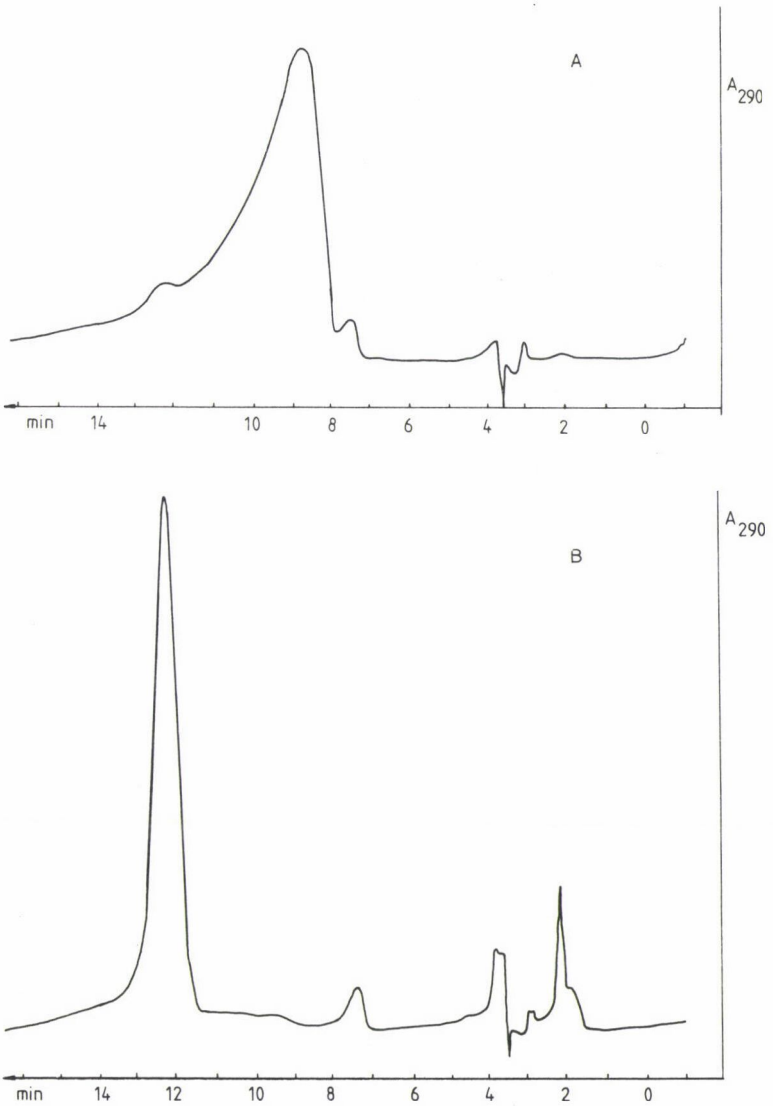

Fig. 6. Transformation of DIMBOA into MBOA, analysis of the two compounds by means of HPLC. *A:* control without heat treatment (in the presence of NH_4OH); *B:* incubated at 60 °C for 5 minutes in the presence of 0.03 mol/L NH_4OH, stored in ice-water until measuring

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OCCURRENCE AND PHYSIOLOGICAL ROLE OF BENZOXAZINONES AND THEIR DERIVATIVES. III. POSSIBLE ROLE OF 7-METHOXY-BENZOXAZINONE IN THE IRON UPTAKE OF MAIZE

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The 7-methoxy-benzoxazinone (DIMBOA) is a compound less toxic for grasses. In glycoside bond its phytotoxicity is moderate. In the roots of two-week-old maize plants grown in culture fluid containing $FeCl_3$, the quantity of DIMBOA-glycoside rises, and the extent of rise is a correlation with the $FeCl_3$ concentration. Iron given in complex form (Fe-EDTA) has no influence on the DIMBOA-glycoside content of roots. Roots of plants grown at different rates of iron supply excrete DIMBOA in a quantity growing parallel with the concentration of iron.

On the basis of the data it is probable that this cyclic hydroxamic acid — similarly to the hydroxamate type siderophores of microorganisms — possesses a phytosiderophore-like function. That is, it may play a role in the Fe^{III} uptake of maize.

Keywords: iron uptake of maize, 7-methoxy-benzoxazinone, phytosiderophores

Introduction

In the tissues of grasses three cyclic hydroxamic acids were detected (Hietala and Virtanen, 1960; Klun et al., 1970; Koyama, 1955; Virtanen et al., 1956; Woodward et al., 1979):

2-4-dihydroxy-2H-1,4-benzoxazin-3[4H]one [DIBOA]

2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3[4H]one [DIMBOA]

2,4-dihydroxy-7,8-dimethoxy-2H-1,4-benzoxazin-3-[4H]one [DIM_2BOA].

Tang et al. (1975) found a considerable amount of these compounds (0.12—2.61 mg/g fresh weight) in the tissues of maize, wheat, rye and Coix lacryma-jobi, while the tissues of barley, oat, millet and rice did not contain any detectable amount of them. It seems therefore, that these cyclic hydroxamic acids (benzoxazinones) do not generally occur in grasses.

The benzoxazinones are present in the tissues in glycoside bond. In the case of tissue destruction (infection, homogenization) the glycosidases release the aglucones. In an aqueous medium the aglucones readily decompose and transform into adequate benzoxazolinones (Virtanen and Hietala, 1959). The benzoxazinones and their derivatives are toxic for certain fungi, bacteria and even insects. They were therefore expected to play a role in pathological resistance, and even in inactivating the herbicides containing 2-chloro-s-triazine as active agent (Hamilton, 1964).

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However, their *in situ* role has not been established. These compounds, which occur in considerable quantities, cannot be present without having some effect.

The hydroxamate type compounds exudated by microorganisms, playing a role in their iron uptake, are called siderophores (Neilands, 1974). Roots of grasses also excrete Fe^{III}-specific complex-forming compounds (Takagi, 1976), and their membranes possess high affinity uptake systems (transport protein), which promote the uptake of compounds forming a complex with the Fe^{III}-ions. Compounds affecting the iron uptake of grasses are called phytosiderophores (Römheld and Marschner, 1986). The phytosiderophores known today are hydroxy- and amino-substituted imino-carboxylic acids, like the mugineic-, and avenic acid (Takagi et al., 1984).

Both the hydroxamate type siderophores and the substituted imino-carboxylic acid type phytosiderophores form a complex on the surface of the cell with iron present in the soil in an oxidized state, and with the help of specific carriers the iron complexes are introduced into the cell where the iron is reduced. Other plants reduce the iron on the surface of the root, and it enters the root in reduced form (Römheld, 1987).

Cyclic hydroxamic acids occurring in grasses (benzoxazinones) also form complexes with Fe^{III}-ions, so it seems reasonable to suppose that these iron complexes may affect the iron uptake of these plants (Tipton and Buell, 1970).

We, therefore, attempted to determine the role of the 7-methoxy-benzoxazinone — the main cyclic hydroxamic acid of maize — in the iron metabolism of maize. For this purpose manifold experiments were set up. On the one hand, in roots of iron deficient plants (grown in Fe-less culture fluid) we studied the effect of iron supply on the 7-methoxy-benzoxazinone content. On the other hand, we examined the 7-methoxy-benzoxazinone concentration in roots of maize plants grown in culture fluids of various iron concentrations. We tried to determine the compound in question in the culture fluid of the plants grown in them, and in the exudate of the roots.

Materials and methods

In the experiments Pioneer 3950 MSc, a two-line maize hybrid was used. The surface of the grains disinfected with NaOCl were germinated at 25 °C for 3 days. The culture fluid experiments were carried out in 1 litre glass cylinders covered with black plastic film. The selected germ plants were placed on a loose plastic net. On the first three days the culture pots contained distilled water, and afterward a culture fluid described by Römheld and Marschner (1986). The plants were grown to the age of two weeks (four-leaf stage) under conditions of constant aeration and 14 hours of illumination. The temperature was 25 °C in the light period and 22 °C in the dark period. Illumination was provided from a distance of 100 cm by ten 40 watt fluorescent tubes. The culture fluid was changed every three days.

The roots of the two-week-old maize plants were rinsed, and for 4 hours every morning they were kept in constantly aerated distilled water. The soaking water was evaporated in vacuum to a small volume and extracted three times with 1-butanol. The dry residue of the butanol phase was chromatographied on Whatman 3MM paper with the upper phase of the solvent ethyl acetate–formic acid–water (60 : 5 : 35). From the $Rf = 0.9$—1.0 region of the chromatogram, the substances were extracted with ethanol, taken up in small volume 50% methanol, and analysed by high pressure liquid chromatography in a Labor MIM type Liquochrom apparatus, on a 25 cm long Chromsil C_{18} reversed phase column. The solvent was 50% methanol, which contained Na acetate of 0.02 mole (pH = 5.6). The flow rate was 1.0 mL/min, detection at 290 nm. The culture fluid of the maize plants was analysed by a similar method.

The fresh plant material was kept for four minutes in a hot water bath. After cooling it was macerated in the presence of quartz sand, then with the boiling water washed over into flasks, and 40 ml ethanol was added to it. The extract was stored at —20 °C until processed. The alcoholic extracts were concentrated to an aqueous phase, and partioner against 1-butanol. The butanolic phase was evaporated dry in vacuum, and taken up in small-volume ethanol. The DIMBOA glycoside was determined with the method of Hamilton (1964). The calibration curve was taken with chromatographically purified DIMBOA glycoside.

The toxicity tests were performed with DIMBOA preparations isolated from five-day-old etiolated plants, after Woodward et al. (1978) and purified chromatographically. From the ethanol-containing stock solution, the required quantities were pipetted into Petri dishes of 10 cm diameter. The ethanol was removed with cold air. The grains to be examined were placed onto one layer of filter paper moistened with water. Germination took place at 25 °C of the thermostat. The germination percentage was established after 48 hours.

Results

Effect of 7-methoxy-benzoxazinone on the germination of grains

In biological tests we found (Pethő, 1992), that the DIMBOA had a toxic effect on plants. After 18 hours of incubation, the explants became white, having lost their initial minimum betacyanin contents. We therefore examined the effect of crystalline preparations isolated from maize plants on the germination of various seeds. As seen from Table 1, a relatively high concentration of DIMBOA had only a low toxic effect on the germination of grasses. The germination of the dicotyledonous *Amaranthus caudatus* and *Lepidium sativum* was appreciably inhibited even by a low concentration of DIMBOA. The two liliaceous species are rather similar in sensitivity to the dicotyledonous species.

In an aqueous medium the DIMBOA decomposes while formic acid is released. We therefore studied the inhibition of germination by different concentrations of formic acid, and found that the germination of *Amaranthus caudatus* seeds was

Table 1

Effect of 7-methoxy-benzoxazinone (DIMBOA) on the germination of seeds from various plants

Species	DIMBOA concentration, μg/mL			
	0	100	500	1000
	germination %			
Zea mays	90.0	90.0	100.0	100.0
Hordeum districhon	97.5	97.5	95.0	97.5
Triticum aestivum	98.0	96.0	94.0	90.0
Secale cereale	98.0	96.0	94.0	98.0
Sorghum bicolor	88.3	89.9	92.3	84.5
Sorghum dochna	89.7	91.5	76.0	80.0
Allium cepa	86.3	80.3	75.4	56.0
Allium porrum	73.5	56.7	25.0	16.7
Amaranthus caudatus	99.6	76.5	0.0	0.0
Lepidium sativum	75.6	44.9	6.7	0.0

inhibited even by a 0.01% solution. Considering that in the case of complete decomposition of a DIMBOA solution of 1 mg/mL concentration, formic acid corresponding to about 0.02% is released, in the germination experiments the toxic effect of formic acid released in the course of the decomposition of DIMBOA cannot be excluded. This seems to be confirmed by the result of our analyses with DIMBOA-glycoside.

Table 2

Effect of DIMBOA-glycoside on the germination of Lepidium sativum seeds and on the length of the radicles

DIMBOA-glycoside mg/mL	Germination %	Root length mm
0.0	90.00	26.1
1.0	87.77	15.2
5.0	71.11	7.4
10.0	36.67	5.5

The formic acid is released with the second position carbon atom splitting off. Because it is between hydroxyl on this carbon atom and the glucose that the glycoside bond is established, the release of formic acid is hindered, while in an aqueous medium the DIMBOA-glycoside can be considered more stable.

Lepidium sativum was found to be less sensitive to the DIMBOA-glycoside than to the aglycon itself; even a tenfold concentration of the glycoside is not so toxic as the free aglycon (Table 2).

Effect of iron supply on the DIMBOA-glycoside content of maize roots

In our first experiment series we added Fe-EDTA and $FeCl_3$, respectively, to the culture fluid of plants raised for two weeks on iron deficient medium, and after 48 hours. When, following the iron supply, the chlorosis otherwise appearing on the younger leaves remarkably subsided, we determined the DIMBOA-glycoside content of the roots. The results obtained are shown in Table 3.

As seen from the data, while iron given in complex form (Fe-EDTA) did not practically change the DIMBOA-glycoside content of the roots, the inorganic iron ($FeCl_3$) significantly increased it.

Table 3

Effect of iron supply on the DIMBOA-glycoside content of maize roots after 48 hours

Treatment	DIMBOA-glycoside µmole/g fresh root
Control	1.57 ± 0.11
$5 \cdot 10^{-6}$ mole/L Fe-EDTA	1.62 ± 0.13
$5 \cdot 10^{-6}$ mole/L $FeCl_3$	2.15 ± 0.23

Subsequently, we followed the changes in the DIMBOA-glycoside content of the roots of two-week-old maize plants in response to increasing concentrations of $FeCl_3$ in the culture fluid. The results are seen in Table 4.

According to the evidence of the data, the suboptimal ($5 \cdot 10^{-8}$ mole/L $FeCl_3$) iron supply hardly changed the DIMBOA-glycoside content of the roots, while the latter remarkably increased when the plants grown in a culture medium with nearly optimum Fe content and did not show symptoms of deficiency.

Table 4

DIMBOA-glycoside content of maize roots grown with different rates of iron supply

$FeCl_3$ concentration mole/L	DIMBOA-glycoside μmole/g fresh root
Control (− Fe)	2.08
$5 \cdot 10^{-8}$	2.10
$5 \cdot 10^{-7}$	2.88
$5 \cdot 10^{-6}$	2.79

Then, we tried to isolate DIMBOA exudated by the maize roots. From our earlier examinations (Pethő, 1992) we knew that the DIMBOA, and the MBOA, the compound formed in the course of its decomposition, when in ethyl–acetate–formic acid–water (60 : 5 : 35) solvent were located in the upper region of the paper chromatogran ($Rf = 0.9$—1.0). We therefore evaporated the exudates, purified them in this chromatographic system, eluated the region of the chromatogram near the front, and analysed the eluate with the help of HPLC at 290 nm.

On the chromatogram definite peaks were obtained at retention times corresponding to DIMBOA and MBOA (Fig. 1). There is no doubt, accordingly, that the roots of maize exudate DIMBOA, a part of which in the aqueous medium transforms into MBOA during the preparation procedure.

It is obvious from the figure that the maize roots exudate 7-methoxybenzoxazinone in distilled water. We subsequently determined the quantity of the DIMBOA exudated.

At the beginning of this series of experiments, we already knew that a considerable proportion of the exudated DIMBOA would be transformed into MBOA, so we had to determine the quantities of both compounds. We measured the quantities of DIMBOA exudated by maize roots grown with different iron supplies. The data are given in Table 5.

On the basis of the data it can be established that in the case of a suboptimal iron supply ($5 \cdot 10^{-8}$ mole/L $FeCl_3$) the amount of DIMBOA exudated by the roots hardly changes, compared to that exudated by the roots of plants grown in ironless culture medium. On the other hand, in the case of a more or less optimum iron supply, the amount of DIMBOA exudated by the roots is twice or thrice the amount exudated by the roots of plants deficient in iron. It is surprising that the

amount of DIMBOA exudated by the roots is about tenfold that of DIMBOA-glycoside measured in the roots. On this basis a vigorous DIMBOA synthesis and metabolism is assumed to occur in the roots.

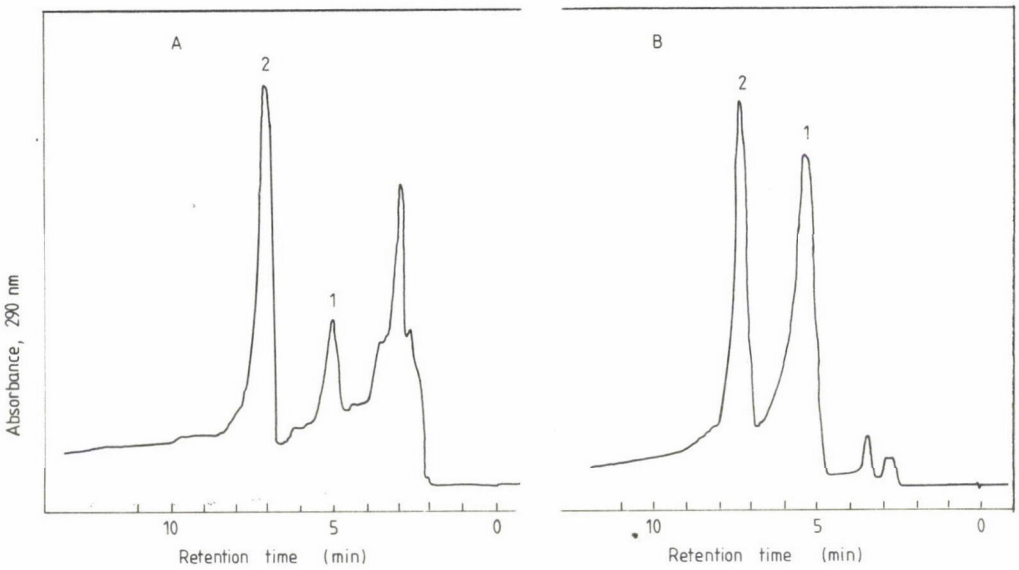

Fig. 1. Analysis of the exudate of maize roots with the aid of HPLC. A: exudate; B: mixture of DIMBOA[1] and MBOA[2]

With the above method we determined the amount of DIMBOA exudated by the roots into the culture fluid during the 3-day culturing period. A part of the compound decomposed again, and with the aid of HPLC we determined the amounts of DIMBOA and MBOA in the chromatographically purified culture fluid. On the basis of the data in Table 6 we concluded that even intact roots (kept in culture medium) may exudate a considerable amount of DIMBOA.

Table 5

Amount of DIMBOA exudated by the roots of maize plants raised with different rates of iron supply between the 2nd and 6th hour after the beginning of illumination

$FeCl_3$ concentration during pre-raising, mole/L	Joint amount of DIMBOA and MBOA in the exudate micromole/g fresh root
Control (− Fe)	11.9
$5 \cdot 10^{-8}$	12.6
$5 \cdot 10^{-7}$	22.9
10^{-6}	28.4
$5 \cdot 10^{-6}$	29.5

On the other hand, it can be seen that the iron supply significantly increases the DIMBOA exudation of roots.

Table 6

Concentration of DIMBOA and MBOA in nutrient solution at different iron supply

Fe supply	DIMBOA + MBOA micromole/L	%
− Fe	180	100.0
$5 \cdot 10^{-7}$ mole $FeCl_3$	260	144.4
10^{-6} mole $FeCl_3$	350	194.4

Discussion

In the course of our experiments we found the grasses to be less sensitive to the 7-methoxy-benzoxazinone than other plants. This is all the more surprising because cyclic hydroxamic acids (benzoxazinone derivatives) have not been detected so far in all grasses. They occur only in certain grass species, but there is no difference in phytotoxic effect between the species, according to whether or not they produce cyclic hydroxamic acids. The 7-methoxy-benzoxazinone is less toxic in glycoside bond (DIMBOA-glycoside).

The fact that the grasses are less sensitive to the compound concerned, and that only the roots of grasses exudate compounds of phytosiderophoric activity which help these plants in taking up iron, may even be an accidental coincidence. However, the experiments show that the iron status of the maize plant influences the DIMBOA-glycoside content of the roots, and the amount of DIMBOA exudated by the roots. These data indirectly prove that in the iron uptake of the maize plant (in its iron metabolism) the cyclic hydroxamic acids (in the present case the 7-methoxy-benzoxazinone) play a part.

Iron introduced in its complex form (Fe-EDTA) does not influence the DIMBOA content. The DIMBOA content and the amount of the exudated DIMBOA rose significantly when the iron was supplied in an oxidized state ($FeCl_3$). It is thus probable that the cyclic hydroxamic acids, like the hydroxamate type siderophores of microorganisms, play a role in the iron uptake; that is, they form another group of the phytosiderophores. The fact that their occurrence is not restricted to the roots, but is found in the shoot as well, suggests that they are synthetized in the leaves (Tipton et al., 1973), but it is also possible that they also participate in the iron transport, and the reduction of iron takes place in the leaves. The question requires further investigations.

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EFFECT OF HEATING AND ULTRA VIOLET LIGHT ON THE FORMATION OF FREE FATTY ACIDS IN THE RICE BRAN OIL

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Heating at 130 °C for 15 or 30 min and illumination by ultra violet light for 15 min were applied on both paddy rice premilling and fresh commercial bran samples. Then, oil was extracted from both sources. Free fatty acid (FFA) content was determined in oils during storage at room temperature for 0—180 days. Heating of either paddy rice or fresh commercial bran inactivated their lipase enzyme and reduced the level of FFAs in the stored oil. In contrast, the ultra violet treatment had no effect. Bran samples were affected by heating more than paddy rice samples. Therefore, it can be recommended to apply heating at 130 °C for 15 min on rice bran to prevent rice brain oil from deterioration during storage.

Keywords: effect of heating, effect of ultra violet light, free fatty acids, oil, rice, rice bran oil

Introduction

Rice mill by-products are bran (7—10%) and germ (2—3%). The rice bran contains about 15% lipids. These lipids are usually affected by the enzymes lipase and lipoxidase, resulting in hydrolytic rancidity and high level of FFAs (Desikachar, 1974), rendering the milled by-products deteriorated during storage. Therefore, it is imperative to stabilize the rice bran and germ so that they can be used for extraction of edible grade oil or for another food and feed purposes.

Among methods for inactivation of the enzymes involved in deterioration of rice lipids, heating excels as it is feasible and practicable for adoption to paddy rice or rice mills (Van-Atta et al., 1952; Tsujino and Kitaichi, 1953; Williams and Beer, 1965; Rothe and Stoeckel, 1967; Virakatamath and Desikachar, 1971; Boling and El-Baya, 1975). Further possibilities are storage under nitrogen atmosphere and at low temperature (Hsieh and Yang, 1969; Yoshikura et al., 1972; Sidhom et al., 1975) and gamma irradiation (Rady, 1981). With regard to evaluation of the obtained oil, very limited work has been reported in the literature. Thus, in the present investigation paddy rice and commercial bran were heat-treated to inactivate lipolytic enzymes and the formation of FFAs in the stored oils were determined during storage.

For the same purpose, effect of irradiation by ultra violet light was also studied.

Materials and methods

Paddy rice samples (variety, Giza 171) were taken from Sakha Research Station, Agriculture Research Center, Ministry of Agriculture, Egypt. Commercial rice bran samples ('Japonica' variety) were obtained from El-Sharkia Rice Milling Company, Zagazig Mills Factory, Zagazig, Egypt.

Each paddy rice sample was divided into four parts, two of them were treated with hot air at 130 °C for 15 or 30 min, cooled, one subjected to ultra violet light for 15 min and the fourth served as untreated control. The treated und untreated samples were milled to obtain the bran.

The commercial rice bran sample was divided into three parts, one was treated with hot air at 130 °C for 15 min, cooled, the another was treated with ultra violet light for 15 min. The last one remained untreated.

The bran samples obtained from paddy rice milling, as well as the commercial bran samples were soaked immediately after treatment in n-hexane and the oils were extracted under vacuum at 30 °C, dried over anhydrous sodium sulphate and stored in brown bottles at room temperature till analyses.

The FFAs% (as oleic acid) was determined after storage of 0, 10, 20, 30, 60, 90, 120, 150 and 180 days according to the method of the A.O.C.S. (1964).

Results and discussion

The oil deterioration during storage is a result of complex chemical and biological reactions and FFA content is used as an index of such deterioration.

The percentages of FFAs (as oleic acid) in the oils extracted from the bran milled from paddy rice, as well as from the bran after treating with high temperature at 130 °C for 15 or 30 min and ultra violet light for 15 min within storage periods of 0—180 days are given in Table 1.

It was suggested that heating paddy rice inactivates lipase enzyme, resulting in the accumulation of FFAs in the oil. After oil storage at room temperature for 6 months, the FFA content of oils extracted from heated samples was less than that of the controls (10.80, 4.58 and 25.38% for 15 min, 30 min heated samples and

Table 1

Effect of treating paddy rice and commercial rice bran with heating at 130 °C and ultra violet (UV) light on the formation of free fatty acids in their stored oils

Storage period of oil (days)	Free fatty acids % as oleic acid						
	Untreated		Heated for 15 min		Heated for 30 min	UV-irradiated for 30 min	
	Paddy rice	Comm. bran	Paddy rice	Comm. bran	Paddy rice	Paddy rice	Comm. bran
0	4.64	14.05	2.90	2.69	1.97	5.45	14.79
10	6.81	18.33	3.35	2.78	2.11	8.38	18.35
20	9.18	22.26	4.23	3.39	2.72	9.83	22.60
30	10.03	23.31	4.60	3.52	2.89	9.86	22.95
60	10.36	25.13	4.66	3.89	2.94	10.01	23.85
90	—	27.05	—	3.91	—	—	25.58
120	10.51	—	5.19	—	3.10	10.29	—
150	—	28.22	—	4.13	—	—	26.36
180	25.38	32.78	10.81	4.21	4.58	25.33	29.98

unheated control, respectively). The oil of unheated rice cannot be stored for long time (6 months) without serious deterioration (FFA content increased from 4.64 to 25.38%). But heating the paddy rice at 130 °C decreased the extent of deterioration even when the oil was stored for 6 months. It was reported that extremely active lipolytic enzymes were the principal factors in bran deterioration during storage (Hunter et al., 1951; Houston et al., 1951; Shaheen et al., 1975) and these enzymes can be inactivated by heating the rice bran at 120 °C for 5—10 min (Tsujino and Kitaichi, 1953) or 100—120 °C for 10—20 min (Srimani et al., 1974).

Heating the fresh commercial bran at 130 °C for 15 min minimized the deterioration of its oil after storage for 6 months (FFA content 4.21%) compared to the unheated sample (32.78%) as shown in Table 1. Here again there is a resemblance between the FFA content of paddy rice and commercial bran oils as heating reduced their FFA content quite considerable compared to the unheated samples. However, FFA data of the unheated samples of paddy rice were lower than those of the commercial bran. This may be due to the longer time of the commercial bran in the laboratory. Therefore, lipase activity might undergo greater changes in the bran than in the paddy rice.

Moreover, the FFA figures of the 15 min heated samples of paddy rice were higher than those of bran. This can be explained by a more potent inactivation of lipase in the bran than in the paddy rice, as the hull inhibits direct heat influence on lipase, partly protecting the enzyme activity.

Exposure of both premilling paddy rice and fresh commercial bran to ultra violet light for 15 min did not influence fat deterioration (Table 1). Thus, it is probable that rice and bran lipase are not sensitive to the ultra violet light or protected by some cell compounds.

Conclusions

Among the methods for controlling accumulation of FFAs in the oil extracted from rice by-products, heating of these materials at 130 °C for 15 or 30 min is feasible and practicable. However, based upon the analytical data of this investigation, heating of fresh commercial rice bran was more effective than that of paddy rice before milling. It can be recommended to apply heating of fresh bran and not on paddy rice, because heat treatment of paddy rice is more expensive, needs more energy and a larger space, since bran amounts to not more than 10% of paddy rice.

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FATTY ACIDS AND UNSAPONIFIABLE MATTER COMPOSITION OF RICE OIL AND RICE GERM OIL AS WELL AS THEIR DEWAXED RESIDUE

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Gas liquid chromatography (GLC) technique was applied for determination of fatty acid and unsaponifiable matter composition of oils extracted from crude rice bran, crude rice germ and dewaxed oils. The results showed a resemblance in the fatty acid contents of each rice bran oil and rice germ oil with those of their dewaxed oils. The major fatty acids in all tested samples were palmitic (22.44—26.16%), oleic (34.37—38.46%) and linoleic (38.24—40—42%), whereas the minor were myristic (0.07—0.12%), stearic (0.29—0.51%) and linolenic (0.19—0.36%). Unsaponifiable matter composition showed that rice germ oil contains 20 compounds and its dewaxed oil contains 19 compounds. Twenty of these fractions were: 14 hydrocarbons, cholesterol, campesterol, stigmasterol, B-sitosterol, $\Delta 5$-avenasterol and $\Delta 7$-avenasterol. The major sterol compounds in rice germ oil and its dewaxed oil were B-sitosterol (50.39%), $\Delta 5$-avenasterol (19.04%), campesterol (13.58%) and stigmasterol (5.46%).

Keywords: fatty acids, oil composition, rice, rice oils, unsaponifiable matter

Introduction

Rice is a staple food for millions of people worldwide. The total annual production of rice is about 450,000,000 tons (Internat. Stat. Yearbook, 1989). Rice plants are threshed to produce paddy rice which is then separated by polishing machine into bran (7—10%), germ (2—3%) and polished rice (87—90%). Bran and germ are used for feeds and oil products (such as edible oil, soap stock and emulsifier for asphalts). Potentially rice bran contains about 11—17% oil, of which 3—9% is wax. Waxes in rice bran oil are widely used as components of various polishing agents, foodstuffs, cosmetics, carbon paper, printing inks, finishing materials and other industrial products. Accordingly, rice bran as a by-product is a rich source of industrially important lipids. Moreover, manufacturers of waxes for either home and industrial uses are interested in obtaining new sources of hard vegetable waxes other than carnauba. With regard to rice germ oil and waxes, very little work has been reported in the literature. Therefore, the present investigation is carried out to determine the fatty acids and composition of unsaponifiable matter of rice bran oil, rice germ oil, as well as their dewaxed oils.

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Materials and methods

Commercial rice bran and rice germ samples (variety *Japonica*) were obtained from El-Sharkia Rice Milling Company, Zagazig Mills Factory, Zagazig, Egypt.

Waxes of rice bran oil and crude rice germ oil were separated according to the method of Kim (1966).

Gas liquid chromatography (GLC) was applied to determine the fatty acids and the unsaponifiable matter composition in oils under investigation. A Pye Unicam gas chromatograph model GCV equipped with a dual channel flame ionization detector was used.

The fatty acids of rice oils were separated as described in A.O.A.C. Methods (1975). The methyl esters of fatty acids were prepared for GLC analysis as reported by Sreenivasan (1968). Fatty acid methyl esters were injected in the glass column (1.5 m) with outer diameter of 4 mm, and packed with 10% polyethylene glycol adipate on Chromosorb W, using a Hamilton microsyringe. The operation was carried out by temperature programmed analysis, the initial column temperature rising was 180 °C. The detector temperature was 250 °C and the analysis time was 45 min. The nitrogen, hydrogen and air flow rates were 30, 33, and 330 ml/min, respectively. The chart speed was 1 cm/2 min and attenuation was 50×10^{-2}.

The fatty acid composition was calculated as the peak area measured by triangulation according to Sreenivasan (1968).

The unsaponifiable matter of oils was prepared for GLC analysis as described by Itoh et al. (1973). It was injected into the glass coiled column (1.5 m) with outer diameter 4 mm, and packed with 1.5% OV-17 on Diatomite C (100—120 mesh) using a Hamilton microsyringe. The operation was carried out by temperature programmed analysis, the initial column temperature was 70°C, the temperature rising rate was 10 °C/min and the final column temperature was 270 °C. The detector temperature was 300°C and the analysis time was 35 min. The nitrogen, hydrogen and air flow rates were 30, 33 and 330 ml/min respectively. The chart speed was 1 cm per 2 min and attenuation was 32×10^{-2}.

The unsaponifiable matter constituents of the samples were identified from the relative retention time of standard pure authentic samples under the same conditions. The unsaponifiable matter composition was calculated using the peak area according to Nelson et al. (1970).

Results and discussion

Fatty acid composition

Fatty acid composition of rice bran oil, rice germ oil before and after wax removal is shown in Table 1. It is clear that rice bran oil, rice germ oil and the corresponding dewaxed oils have a similar fatty acid composition. The major fatty acids in all tested samples were palmitic, oleic and linoleic acids. The calculated means were 23.70, 36.54 and 39.06% for palmitic, oleic and linoleic respectively. The minor fatty acids were myristic, stearic and linolenic acids. The means were 0.10, 0.38 and 0.26% for myristic, stearic and linolenic acids respectively. This trend was in a good agreement with those reported previously (Izzo et al., 1972; Resurreccion and Juliano, 1975; Choudhury and Juliano, 1980; Tanaka et al., 1982). Somewhat different data of fatty acid composition of rice bran oil were published by other investigators (Cocks and Rede, 1966; Sandler et al., 1968; IRRI, 1973; Yokochi, 1974; Khan, 1979; Gaydou and Raonizafinimanana, 1980), this may be due to the genotype of rice variety and environmental or seasonal conditions.

Unsaponifiable matter composition

The data of GLC analysis are given in Table 2. Twenty compounds were detected in the unsaponifiable matter of germ oil, whereas 19 compounds were

detected in the unsaponifiable matter of its dewaxed oil. The detected compounds in the unsaponifiable matter of germ oil are similar to those found in its dewaxed oil. Hydrocarbons C_{12}, C_{13}, C_{16} and C_{27} were not found in that of germ oil, whereas

Table 1

Fatty acid composition of oil and dewaxed oil from rice bran and rice germ

Fatty acid	Rice bran		Rice germ	
	oil %	dewaxed oil %	oil %	dewaxed oil %
Myristic ($C_{14:0}$)	00.12	00.12	00.08	00.07
Palmitic ($C_{16:0}$)	22.62	22.48	23.53	26.16
Stearic ($C_{18:0}$)	00.29	00.36	00.51	00.37
Oleic ($C_{18:1}$)	38.46	38.23	35.10	34.37
Linoleic ($C_{18:2}$)	38.24	38.49	40.42	38.87
Linolenic ($C_{18:3}$)	00.26	00.23	00.36	00.19
Total saturated	23.03	23.05	24.12	26.60
Total unsaturated	76.96	76.95	75.88	73.40

Table 2

Unsaponifiable matter composition of rice germ oil and its dewaxed oil

Components		RRT*	Oil %	Dewaxed oil %
1. n-dodecane	C_{12}	0.07	—	0.21
2. n-tridecane	C_{13}	0.10	—	traces
3. n-hexadecane	C_{16}	0.23	—	0.23
4. n-octadecane	C_{18}	0.34	traces	0.16
5. n-eicosane	C_{20}	0.37	0.13	0.18
6. n-heneicosane	C_{21}	0.43	0.24	—
7. n-doeicosane	C_{22}	0.46	3.28	traces
8. n-tetraeicosane	C_{24}	0.51	0.15	traces
9. n-hexaeicosane	C_{26}	0.56	0.10	traces
10. n-heptaeicosane	C_{27}	0.58	—	0.15
11. n-octaeicosane	C_{28}	0.60	1.53	0.37
12. n-triacontane	C_{30}	0.65	1.72	1.87
13. squalene		0.69	0.44	0.37
14. unknown (1)		0.72	0.18	—
15. unknown (2)		0.73	0.11	—
16. n-dotriacontane	C_{32}	0.75	0.17	—
17. cholesterol		0.80	0.41	0.30
18. unknown (3)		0.82	1.75	0.57
19. campesterol		0.88	10.71	16.44
20. stigmasterol		0.93	4.95	5.96
21. B-sitosterol		1.00	52.38	49.39
22. unknown (4)		1.06	3.27	3.13
23. Δ5-avenasterol		1.15	17.48	20.59
24. Δ7-avenasterol		1.36	0.95	—

* Relative retention time for B-sitosterol (retention time 34 min) was taken as 1.00.

hydrocarbons C_{21} and C_{32} were not found in that of its dewaxed oil. Comparing the composition of hydrocarbons of the two oils, n-paraffins are distributed more evenly. The presence of the relatively low carbon number hydrocarbons are found in higher quantities in dewaxed germ oil than in germ oil.

The predominant sterol in the two oils is B-sitosterol which was found in larger amount in germ oil than in its dewaxed one. Dewaxed oil contained higher value of $\Delta 5$-avenasterol than that of germ oil. The data obtained for sterols are in a good agreement with those given by Kuroda et al. (1977) who found that B-sitosterol, campesterol and $\Delta 5$-avenasterol were the major sterol components in the rice bran oil, whereas cholesterol was the minor one. Similar observations were reported by other investigators (Itoh et al., 1973; Gaydou and Roanizafinimanana, 1980) for rice bran oil with excepton to $\Delta 5$-avenasterol.

Conclusions

During rice processing, a substantial amount of by-products forms. These milled products easily decompose during storage due to lipolytic and oxidative changes of their lipids by the enzymes lipase and lipoxidase, concentrated in the bran layers and germ of rice kernels. To avoid this, the oil can be extracted from the by-products. At the present time, the rice bran oil and rice germ oil are mainly used for industrial purposes. Unfortunately, the rice oils undergo also an extremely rapid deterioration. However, this can be prevented by some treatments either on paddy rice or on milled by-products, such as subjecting to steam or moist air at high temperature and storage under nitrogen atmosphere and at low temperature.

As the analytical data (Tables 1 and 2) show, these oils are suitable for food or feed purposes also, having high content of unsaturated $C_{18:1}$ and $C_{18:2}$ fatty acids (about 77%) and low content of cholesterol (0.41%).

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STUDY ON THE ROOT DEVELOPMENT OF ASPARAGUS (*ASPARAGUS OFFICINALIS* L.)

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The asparagus is an indigenous plant in Hungary. Its cultivation is economical, but for the subsequent plantations a new method of seedling raising is required. We studied the location of the root system of older plants in the soil with a view to modernizing the cultivation.

The seeds were sown in propagation boxes, then the seedlings transplanted in 5 l containers and raised until the end of the vegetation period. We studied the possibility of direct sowing into 250 cm^3 paper cups, for which the seed was kept wet at 22 °C for 92 hours.

We studied the root development of a commercial asparagus plantation.

The formation of a strong spindle root is demonstrable in the first phase of seedling raising.

The root development was stimulated by the combination of peat, organic manure and zeolite. There were considerable differences between the varieties in the average root weight of two-year-old plants, the same was found for the number and length of the spindle roots.

More than 50% of the root system of the bearing asparagus plantation was located in the 20—40 cm layer of soil. The proportion of necrotized roots was largest in the 20—40 cm soil layer. Most of the roots were located in the row and toward the row space they gradually decreased in number.

On the basis of the results obtained, we gained important information for the modernization of asparagus growing in Hungary, which we should like to use in scientific work and in practice.

Keywords: *Asparagus officinalis*, root type, root weight, rhizome weight, soil mixture, zeolite

Introduction

The asparagus (*Asparagus officinalis* L.) is an indigenous vegetable in Hungary which occurs wild even in these days. Its cultivation goes back centuries; its description as a fine vegetable, as well as its cultivation technology are to be found in Lippay (1664). In western countries of Europe, in Taiwan, Japan and North-America asparagus is a popular, but expensive kind of vegetable. Authors are much concerned with its botanical description, cultivation and possibilities of yield increase. The asparagus is a marketable vegetable of high price, to urge its cultivation in Hungary is therefore, fully justified. If the yields are to be so high as to make the cultivation of asparagus economical, thorough knowledge must be acquired of the development phases of asparagus under the ecological conditions of Hungary. With this in view we carried out studies on the root development of asparagus, and in possession of the results we should like to improve the present seedling raising technology, soil preparation and nutrient replacement techniques.

On the seedling of the asparagus the cotyledon, the main root and rudiments of later roots as well as the primary stem can be well distinguished. The root of the asparagus is organized in the apical meristem in the course of ontogeny and the abstriction of the other tissues starts from a single initial group (Terpó, 1986). The asparagus has a fibrous root-system which consists of two root types: storing- and sucking roots. The thickness of the cylindrical, non-branching, continuously growing roots may come close to 10 mm (Bruno, 1984). A part of the root-system penetrates vertically into the soil to a depth of 2.0—2.5 m (Dávid and Koródi, 1969), or sometimes even 3—4 m (Franken, 1968). The rooting capacity of the asparagus plant was studied by Yakuwa et al., (1982), who found that the bulk of the total root-system of a bearing plantation was located in the 0—40 cm soil layer, and only 8—11% penetrates deeper than 40 cm.

The rhizodermis, the dermal tissue of the root functions at the same time as an absorbing tissue, and is typically one-layer thick in the asparagus too (Terpó, 1986). According to Fehér-Barvinkó (1987) an asparagus seedling 180 days old raised in a 1 litre container possessed 19—31 6.5—40 cm long storing roots, while those of a two-year-old plant were 54—113 in number and an average of 275 cm long. Hahn and Zell (1978) and Fehér-Barvinkó (1981) found that the time of raising seedling in container can be reduced by one year. Benson (1982) describes a seedling raising method by which the seedlings are suitable for planting out in 10—12 weeks. According to Fischer and Benson (1983) in six weeks of seedling raising plants with five 26—28 cm long shoots and 9—11 storing roots, suitable for planting out can be obtained. Dufault and Waters (1984) reported on favourable results attained by planting out 10—12 weeks old seedlings.

Materials and methods

The root formation of the asparagus plant was examined in specimens of different development stage and age.

In the case of raising seedlings under controlled conditions the seed was sown to rows in propagation boxes, then the seedlings were transplanted in 5 l containers where they were kept until the end of the vegetation period. During the vegetation the plants were treated on three occasions with 1.0% Volldünger nutritive solution (1 dl/container).

For direct sowing in 250 cm^3 paper cups the seed was kept wet over 92 hours at 22 °C, then placed one by one 1 cm deep in the soil. Sowing was made in a culture medium prepared from a mixture of 2/3 part peat, 1/3 part mature farmyard manure, 1.5 kg/m^3 Plantozán fertilizer. The average soil temperature on sowing was 17.9 °C (minimum 14.4 °C, maximum 28.4 °C).

An experiment was set up with Franklim F-1 hybrid to examine the effect of 30 cm^3 of various soil mixtures. In this case the seed was soaked in water for 48 hours at room temperature. Sowing was carried out seed by seed in glasshouse on 20th April.

Root development was studied in a two-year asparagus stand planted under polythene in sand at a spacing of 50 × 40 cm, as well as in a young asparagus plantation under commercial production. The root-system of the plants raised in plastic house was washed out with water. In the commercial plantation the roots were extracted by screening from a 20 × 20 × 20 cm volume of soil.

From the young seedlings 5 plants, from the container stand 20 plants, from the plastic house plantation 10 plants per measuring date, and in the commercial plantation 3 × 1 and 5 × 0.8 m soil, were examined for root development. In the experiment nine asparagus varieties were included.

The results of measuring were evaluated using mathematical methods.

Results

According to the results of root-system studies on asparagus hybrids, as regards the development of the main roots of seedlings, there was no essential difference between the dates of measuring, but the number of roots per plant was in each case more than in the offsets. The faculty of forming strong spindle root is demonstrable for the aspragus as early as in the first phase of seedling raising. According to the measuring data the largest number of rootlets were formed on the main root of the hybrid Geynlim F-1 on each occasion of measuring. The ratio of main root to rootlet rose from 18.8 to 21.7 on an average during the period examined. The number of rootlets per plant increased by 141.1 on an average of the hybrids and plants. Of the hybrids examined, Geynlim F-1 showed the most intensive and Backlim F-1 the poorest root formation (Table 1).

The length of rootlets per seedling grew multiple in 25 days in each hybrid. Significant differences in rootlet formation between the hybrids were obtained on 9th May. The spindle root character of the main root develops gradually with the age of the seedling; the rootlets became thicker and an ever increasing number of secondary rootlets formed. This was evident on the 6th June occasion of measuring, when the average length of secondary rootlets per plant was 4.193 mm ranging from 2878.2 to 5237.8 mm. Thus, according to the examinations a seedling with a 22.5 cm long stem and 4.1 offsets has an absorbing root-system of 4.19 m.

On two occasions, measuring showed significant differences in root weight between the hybrids. Geynlim F-1 had a significantly larger root weight (28th April, 9th May) than either Backlim F-1 or Franklim F-1. According to the last measuring data the root weight showed a wide scatter, so they cannot be regarded as evaluable.

On the first three occasions of measuring, the total weight of the asparagus hybrids gave in each case a significant result for the fresh weight. On the basis of the total weight considerable differences in growth were found between the hybrids. Of the hybrids examined Geynlim F-1 and Venlim F-1 showed particularly vigorous growth.

The root development of Franklim F-1 seedlings raised in different soil mixtures of 30 cm^3, when analysed from various aspects showed no essential differences in the initial phase of development, though the effect of the culture medium was visible in the length of the main root and the number and length of the rootlets per plant compared to the average of the treatments (Table 2).

The second survey was made on the 90th day after sowing (16th July), when essential differences were found in the root formation. According to the results of measuring the culture media containing zeolite and alginite, respectively, stimulated the growth of the main root and rootlets. The 70-day seedlings raised in 30 cm^3 culture medium reached a development stage suitable for planting out and had a strong root-system. The spindle root formed by then was 23.7 mm and the absorbing roots 91 mm long per 10 mm length of shoot.

According to the examination results of the root development of one- and two-year asparagus plants (Table 3) the average root weight in the variety Viking KB3 reached in one year 70.3% of the root weight of two-year-old plants. The

Table 1

Root development of asparagus seedlings

Designation	Time	Geynlim F—1	Backlim F—1	Franklim F—1	Venlim F—1	Average
Root system	12. IV.	2.2	2.0	2.4	2.0	2.15
main root	28. IV.	3.4	2.6	3.4	3.6	3.25
n/plant	9. V.	4.2	3.6	4.8	4.4	4.25
	16. IV.	7.2	8.0	8.8	8.4	7.35
length of main root	12. IV.	234.8	182.0	216.4	210.2	210.80
mm/n	28. IV.	331.4	177.8	284.4	307.2	260.20
	9. V.	421.0	326.8	524.8	507.6	445.00
	16. VI.	696.6	712.6	811.4	791.6	753.00
rootlet n/plant	12. IV.	56.0	41.2	40.6	46.6	46.10
	28. IV.	106.4	60.2	71.6	99.4	84.30
	$LSD_{5\%}$	30.69				
	F	4.90				
	9. V.	170.4	137.0	137.0	131.6	144.00
	16. VI.	203.0	123.0	190.4	124.4	160.20
rootlet mm/plant	12. IV.	995.4	710.0	786.0	970.6	865.30
	28. IV.	1167.6	676.0	1095.2	1448.0	1096.70
	9. V.	1722.8	1522.4	2299.6	2451.2	1998.70
	$LSD_{5\%}$	543.70				
	F	6.405				
	6. VI.	2794.4	1823.6	2688.8	2742.6	2512.30
secondary rootlet	16. VI.	1418.0	1054.6	1755.0	2495.2	1680.70
length mm/plant	16. VI.	4212.4	2878.2	4443.8	5237.8	4193.05
total mm/plant						
root weight	28. IV.	334.0	202.0	238.0	334.0	277.00
mg/n	$LSD_{5\%}$	89.55				
	F	5.384				
	9. V.	786.0	344.0	604.0	775.0	527.20
	$LSD_{5\%}$	202.69				
	F	9.845				
	16. VI.	2546.0	2598.0	5660.0	3708.0	2853.00
air dry weight	28. IV.	129.0	113.0	123.0	99.0	116.0
mg/n	9. V.	142.0	114.0	196.0	174.0	156.50
Total weight	12. V.	492.0	308.0	400.0	510.0	427.50
mg/plant	$LSD_{5\%}$	133.62				
	F	4.641				
	28. IV.	1202.0	558.0	916.0	1210.0	971.50
	$LSD_{5\%}$	316.26				
	F	8.989				
	9. V.	1235.0	849.0	1187.0	1621.0	1223.00
	$LSD_{5\%}$	349.87				
	F	7.749				
	16. VI.	4128.0	3998.0	4506.0	6504.0	4784.00

average number of spindle root per plant was 90.2% for one-year plants compared to two-year ones; accordingly, the number of storing roots showed less differences than their weights.

Table 2

Effect of the composition of culture medium on the root development of seedlings Franklim F—1
Cubic volume of the nutrient cylinder: 30 cm^3

Composition of culture medium	Root system									
	Main root				Rootlet				Secondary rootlet	
	n/plant		mm/plant		n/plant		mm/plant		mm/plant	
	14. VI.	6. VII.	14. VI.	6. VII.	14. VI.	6. VII.	14. VI.	6. VII.	14. VI.	6. VII.
1. Peat 50% Organic manure 50%	2.8	5.8	84.8	207.7	18.1	50.6	125.5	599.4	12.4	299.6
2. Peat 25% Organic manure 50% Sand 25%	2.8	3.9	101.7	169.3	31.3	47.0	194.7	510.6	19.5	146.3
3. Peat 25% Organic manure 50% Alginite 25%	3.2	6.9	115.2	293.4	23.8	78.9	187.1	834.0	17.3	276.5
4. Peat 25% Organic manure 50% Zeolite 25%	3.2	5.9	120.5	279.1	27.7	71.8	264.2	737.0	43.7	240.0
Average	3.0	5.6	105.5	237.3	25.2	62.0	192.8	670.2	23.2	240.4
$LSD_{5\%}$	—	1.37	—	74.74	—	16.78	—	183.22	—	3.68
F	—	7.04	—	5.22	—	7.33	—	4.90	—	3.91

The average total length of storing roots was 56.9 cm for the one-year asparagus plants, less — 96.6% — than in the case of two-year plants. In the average length of spindle roots the difference was only 1.13 cm between the two age groups (Table 3).

The average weight of the two years old asparagus plants varied from variety to variety. The variety H-3 showed the most vigorous development which appeared in the number and length of spindle roots per plant and the average length of root too. The Backlim F-1 hybrid was planted outdoors at a plant distance of 40 cm in one row, while the other varieties were spaced at 40×40 cm in plastic house. According to the results of examinations the root development of the two-year-old asparagus plants was better influenced by the variety than by the spacing (Fig. 1).

The root weight of two-year asparagus plants raised under polythene at a spacing of 40×40 cm ranged between 1103.1 and 1822.5 kg per 1000 m^2, while the corresponding value for the Backlim F-1 hybrid grown under field conditions was 1849.3 kg.

We exposed the root-system of a four-year plantation of the variety Eros grown under farm conditions in order to determine the distribution of roots in the row and row space. The soil of the 2 m^2 model area was divided into blocks of $0.2 \times 0.2 \times 0.2$ m, screened, and the roots weighed; then the intact and injured roots were numbered.

Table 3

Root characteristics of one- and two-year asparagus plants

Designation	Unit	Viking KB 3		H—1	H—3	UC—157	Boklim F—1
		1	2	3	3	3	4
Av. weight of plants	g	176.5	124.2	277.7	291.6	231.9	295.9
minimum	g	83.0	68.0	194.0	175.0	159.0	190.0
maximum	g	344.0	202.0	378.0	414.0	346.0	458.0
Spindle root av.	n/plant	82.8	74.2	74.7	109.4	62.3	82.1
minimum	n/plant	12.0	49.0	56.0	87.0	49.0	57.0
maximum	n/plant	122.0	109.0	92.0	146.0	88.0	107.0
Total length of av. root	cm/plant	1716.8	1659.9	1920.9	2973.7	1317.4	2054.5
minimum	cm/plant	1052.0	891.0	1384.0	1607.0	932.0	1314.0
maximum	cm/plant	2210.0	1997.0	2592.0	5117.0	2033.0	2453.0
Spindle root av. length	cm/n	20.53	19.4	25.5	26.6	21.0	25.4
minimum	cm/n	13.9	17.7	22.0	20.0	19.0	20.0
maximum	cm/n	25.8	21.9	28.3	35.1	23.1	28.9

1. Planted in polythene house on 13 May 1988
2. Planted in polythene house on 10 May 1989
3. Planted in container on 27 April 1988, in polythene house on 27 July
4. Planted in the field on 27 April 1988

According to the results of the examinations 38.6% of the root-system of the asparagus plants were located in the 0—20 cm, 54.7% in the 20—40 cm and 6.7% in the 40—60 cm soil layer (Table 4).

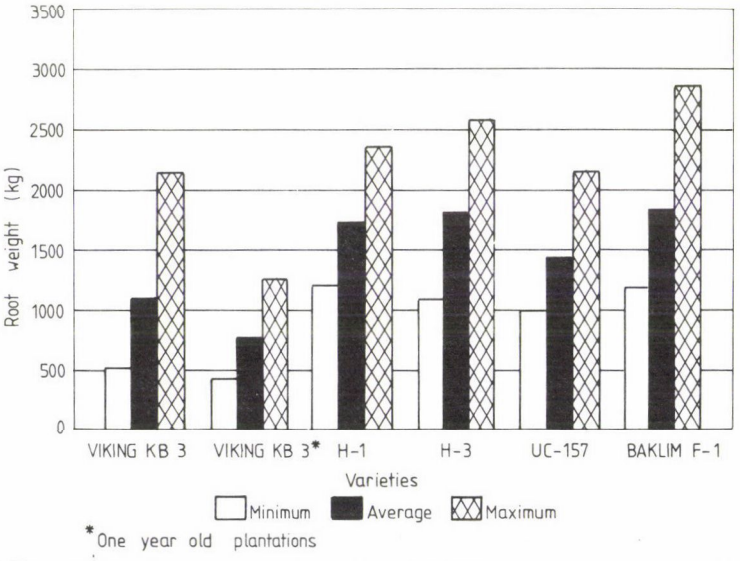

Fig. 1. Root weight of an asparagus plantation with different spacings, kg/1000 m^2

The largest proportion of necrotized roots was found in the 20—40 cm soil layer; the weight of the absorbing roots was 2.0% of the total weight of the root-system.

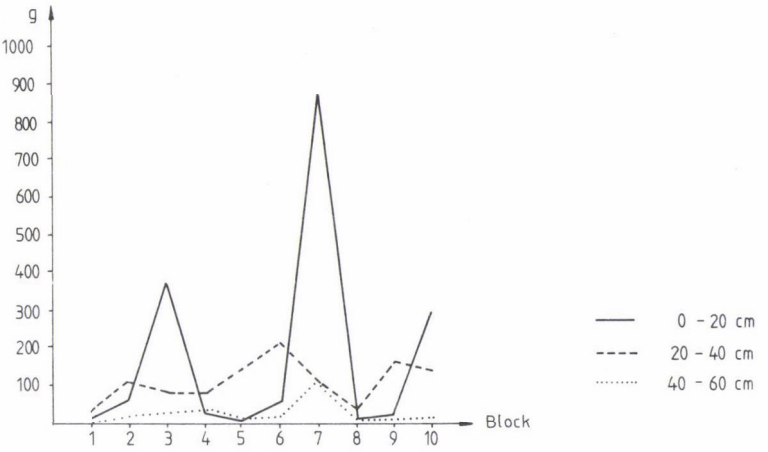

Fig. 2. Root weight in row and row space

The root weight of the blocks (Fig. 2) and the number of roots (Fig. 3) were largest in the row, and steadily decrease with the distance from the row. The distribution by depth showed a similar tendency. The roots were located more or less concentrically both in the row and in the row space. The asparagus roots grow at angles to the main stem, radially in the direction of the lower level and the row.

Table 4

Rhizome- and root weight per 1000 m^2, kg

Designation	Depth			
	0—20 cm	20—40 cm	40—60 cm	Total
Rhizome weight	644	—	—	644
Total root weight	751	1071	135	1957
% of total	(38.6)	(54.7)	(6.7)	(100.0)
Storing root weight	720.2	941.7	133.0	1661.9
Necrotized root weight	11.4	115.7	1.25	128.3
Rootlet (absorbing) weight	19.4	13.6	7.7	40.7

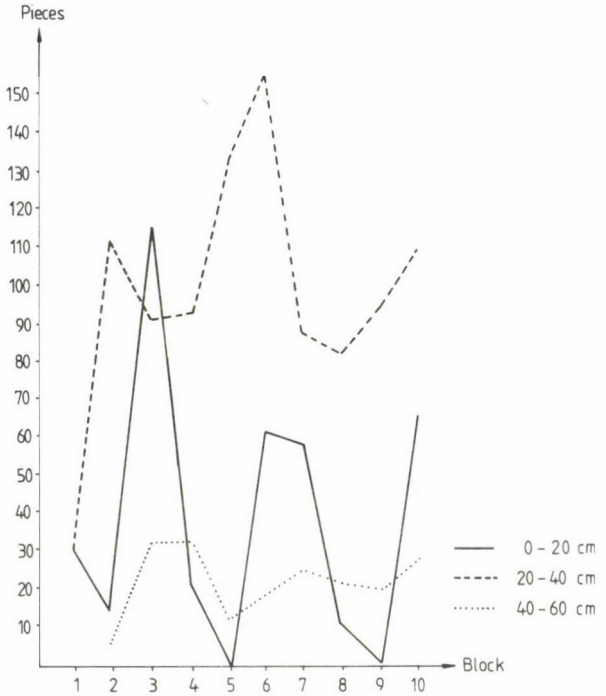

Fig. 3. Number of roots in row and row space

Discussion

The examinations following the emergence of the asparagus seeds unequivocally confirmed Terpó (1986) who found that even in the initial phase of development the seedlings showed the two types of root. The results of our observations of the cylindrical, continuously growing roots were similar to those of Bruno (1984), namely, they do not branch off; but according to our measuring data the thickness of the spindle roots did not even in the four-year plantation approach the 10 mm diameter. We found that the 4—5 mm root diameter was typical. In younger plants storing roots thinner than that were formed. With the ageing of the plant the storing roots probably increase not only in length but also in thickness.

In the course of examining the asparagus plants we do not find the rooting depth reported by Dávid and Koródi (1969) and Franken (1968) for four-year-old farm-scale plantations. This may also be connected with the fact that we studied the root-system in young plantations. According to our examinations in some cases asparagus roots were not even present as deep as 60 cm.

Yakuwa et al. (1982) found the bulk of the root-system to occur in the row and row space in the 0—40 cm soil layer in the case of twelve-year-old plantations. This statement is partly supported by our own examination results, which show that the

lowest number of roots are located in the 40—60 cm soil layer, while 20—40 cm deep more roots are present than in the uppermost layer of soil.

This result can be explained by the fact that the root formation of the asparagus plants tend towards the soil surface from year to year. The young plantation we studied did not show much of this phenomenon. From this we can draw the conclusion that in Hungary regular application of organic manure at a depth of 40—50 cm is necessary, since the bulk of the storing roots and the absorbing root-system formed on them are located at that level.

A similar result was obtained in the rows and row spaces for the tendencies of root development and root destruction. Our examinations confirmed the result obtained by Hahn and Zell (1978), namely, that with the container method the period of seedling raising can be reduced by one year. Our results agreed with those published by Fischer and Benson (1983), Dufault and Waters (1984): in 10—12 weeks seedlings suitable for planting out can be raised.

Summary

Asparagus officinalis L. can be grown rather economically, still, to improve its economic efficiency we studied the development of the root-system from the time of emergence.

In the post-emergent period no essential differences in root development were found between the varieties examined. As regards the absorbing roots (rootlets) the most intensive growth was shown by the Geynlim F-1 hybrid.

By the time the asparagus plants developed an average of 22.5 cm shoot (6th June) and formed 4.1 offsets, the absorbing root-system reached a total length of 419 cm.

According to the examination results of the total weight of asparagus hybrids, significant results were obtained in all cases concerning the fresh weight (Table 1).

The root development of seedlings raised in nutrient cylinder of 30 cm^3 was stimulated by the medium containing zeolite and alginite, respectively; 90 days after sowing seedlings suitable for planting out developed (Table 2).

The average weight of one- and two-year asparagus plants ranged from 124.2 to 295.9 g, the number of spindle roots from 74.2 to 109.4 per plant, the total length of the storing roots from 1317.4 to 2973.7 cm, and the average length of the spindle root between 19.4 and 26.6 cm (Table 3).

The weight of roots in terms of 1000 m^2 was 1103.1—1822.5 kg depending on growing space and variety (Table 1).

Of the roots of a four-year-old bearing plantation 38.6% were located in the 0—20 cm, 54.7% in the 20—40 cm, 6.7% in the 40—60 cm soil layer. The proportion of necrotized roots was 2% (Table 4).

The weight and number of roots located in the row and row space, respectively, are shown in Figs 2 and 3.

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ESTIMATES OF GENETIC PARAMETERS FOR YIELD OF SIX MAIZE INBREDS AND THEIR CROSSES IN LOW AND HIGH PLANT DENSITIES

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In this study six maize inbreds and their 15 single crosses, including six generations of each cross, i.e. P_1, P_2, F_1, F_2, Bc_1 and Bc_2 were used. The performance of grain yield per plant were determined under two population densities, i.e. 20 and 30 thousand plants/fad. Estimation of the type of gene action and degree of dominance, controlling grain yield were obtained from individual plants within segregating generations. The results indicated that the correlation coefficient between the two plant densities was highly significant for grain yield of all genotypes studied. Increasing plant density from 20 to 30 thousand plants/fad. tended to decrease grain yield for the single crosses, whereas it was fluctuated and had insignificant effects for the parental lines. Highly significant estimates of heterosis and inbreeding depression were found for grain yield in the 15 crosses.

Estimates of additive and dominance variances were important sources for the genetic variability of grain yield per plant in all single crosses under this study. The average degree of dominance was in ranging from partial to over dominance. Therefore, the estimates of additive and dominance variances might be used in the planning of prospective breeding program using the advanced generations of the 15 crosses as a source of deriving and selecting new inbred lines having high general combiners of grain yield for developing new hybrids having good specific combining ability and heterosis among their parental lines in the future hybrid production program.

Keywords: genetic parameters, grain yield, maize, plant density

Introduction

Several informations are published on the interaction between the environmental factors and genotypes of maize. Variation in plant population had a significant effect on the productivity (Odiemah, 1973). Grain yield of maize hybrids, planted at high densities are markedly reduced. Therefore, it is important to determine the genotypes that are tolerant to high plant density. In the production of maize hybrid seed, the yield of inbreds is often a limiting factor. So, it seemed appropriate to study the feasibility of increasing the productivity of inbreds through the use of high population densities. However, many investigators found that the broad genotypes (varietal crosses or single crosses) were more stable than narrow basic genotypes (parental varieties or inbreds) under different environmental conditions (Sprague and Federer, 1955; Eberhart and Russel, 1966; Galal et al., 1984).

Maize breeders are interested in estimating the magnitude of genetic variance and type of gene action in their materials because of their implications in choosing the most effective selection and breeding procedures which increase the ability to identify the desired genotypes in the studied populations. The presence of large and significant estimates of additive genetic variance indicates that selection of new superior lines from the segregating generations of a given cross would be possible. On the other hand, the presence of significant estimates of dominance genetic variance suggests that the great advantage would be for the F_1 hybrides performance.

Estimates of genetic variances in maize populations have been studied by several authors (Robinson et al., 1955; Sprague, 1964; Stuber and Moll, 1971). Some studies, however, have been reported for genetic parameters of additive, dominance and epistatic effects for grain yield and other traits in different inbreds of maize (Mariani and Desiderio, 1975; Schnell and Singh, 1978; Odiemah and Oraby, 1986).

Several statistical models have been elaborated to determine the components of genetic variance and its partitioning into additive and non-additive genetic effects, such as proposed by Mather and Jinks (1982).

Accordingly, the objective of this investigation was to study the response of six inbreds and their crosses to increasing densities for grain yield per plant, and to estimate the type of gene action responsible for heterosis, heritability and expected genetic advance in the 15 single crosses of maize, in addition to evaluate these populations as base materials for breeding programs.

Materials and methods

Fifteen single crosses of all possible combinations among six inbred lines of maize, i.e. Kfs_1, Kfs_2, Kfs_3, Kfs_4, Kfs_5 and Kfs_6 were chosen for this study. In 1986 season the 15 single crosses and their parental lines were used in a series of crosses and selfings to produce the different populations. Each single cross was self-pollinated to obtain the F_2's population and back crossed to both respective parents to get Bc_1 and Bc_2. In 1987 the six populations, i.e. female parent (P_1), male parent (P_2), first generation (F_1), second generation (F_2), F_1 back crossed to P_1 (Bc_1) and F_1 back crossed to P_2 (Bc_2) of each cross were grown under two plant densities, i.e. 20 (low) and 30 (high) thousand plants per fad (fad = 4200 m^2). This investigation was carried out at the farm of the Faculty of Agriculture, Tanta University Kafr El-Sheikh, in a complete randomized block design with four replications. Plot size was 15 rows of F_2 plants and three rows for each of the other five populations, 4 m long and 80 cm apart.

Hills were 25 cm apart for the first experiment of low plant density and 17.5 cm apart in the second experiment of high plant density. The recommended culture practices for optimum maize production were followed. Data of grain yield/plant were taken on the plot mean of F_2 generation and the three rows for the other populations. At harvest, grain yield per plant (gm) was recorded as an average of total yield of plants/plot. Grain yield was adjusted to 15.5% grain moisture contents.

Statistical analysis

The analysis of variance for the data of grain yield/plant (gm) was calculated for each of the two experiments. Combined analysis was computed over the two plant densities in the two years for the parents and their single crosses to estimate the genotype by environment interaction. A homogeneity of error test was carried out according to Snedecor and Cochran (1980). The effects of lines and crosses were considered as fixed effects, while the environments were considered to be of random effects.

Heterosis and inbreeding depression were computed as deviations of the F_1 mean from the midparental mean (MP) and the F_2 mean, and expressed as a percent of midparental and F_1 means, respectively.

The population means were tested by using scaling test outlined by Mather and Jinks (1982) to test the adequacy of the additive-dominance model in each case as follows:

$$A = 2Bc_1 - P_1 - F_1 \qquad V_A = 4VBc_1 + VP_1 + V_{F1}$$

$$B = 2Bc_2 - P_2 - F_1 \qquad V_B = 4VBc_2 + VP_2 + V_{F1}$$

$$C = 4F_2 - 2F_1 - P_1 - P_2 \qquad V_c = 16V_{F2} + 4V_{F1} + V_{P1} + V_{P2}$$

Standard errors of A, B and C were obtained as the square root of the corresponding variance. If the data from a cross gave insignificant value of A, B and C the additive-dominance model is perfectly adequate for the analysis of the variation in that cross for the certain trait. It was observed that simple model of Mather and Jinks (1982) was adequate to explain the inheritance of grain yield in the 15 crosses. The variances of the three non-segregating populations; P_1, P_2 and F_1 used to estimate environmental variance as follows:

$$\text{Env. variance } (V_E) = \sqrt[3]{V_{\bar{F}1} \cdot V_{\bar{F}2} \cdot V_{\bar{F}1}}$$

A one-tail F ratio was calculated to test the significance of F_2 variance from environmental variance. Mather and Jinks (1982) were used for the partitioning of the total F_2 phenotypic variance (V_{F2}) into its components, i.e. additive (D) and dominance (H) variances according to the following expectations:

$$V_{F2} = \frac{1}{2}D + \frac{1}{4}H + E$$

$$V_{Bc_1} = \frac{1}{4}D + \frac{1}{4}H + E$$

$$V_{Bc_2} = \frac{1}{4}D + \frac{1}{4}H + E$$

$$\text{Degree of dominance } (\bar{a}) = \sqrt{\frac{2H}{D}}.$$

Broad and narrow senses of heritability (h^2) and expected genetic advance (Δg) were estimated using methods developed by Lush (1984) as follows:

$$h^2 \text{ (broad sense)} = \frac{\frac{1}{2}D + \frac{1}{4}H}{\frac{1}{2}D + \frac{1}{4}H + E}$$

$$h^2 \text{ (narrow sense)} = \frac{\frac{1}{2}D}{\frac{1}{2}D + \frac{1}{4}H + E}$$

$\Delta g = k \cdot h^2 \cdot \sigma$ ph where,

σ ph: phenotypic standard deviation and K; selection differential (1.76 at 10% selection intensity).

The minimum number of genetic factors was estimated by formulae attributed to Sewall-Sright (cf. Burton 1952):

$$n = 0.25\ (0.75 - h + h^2) D^2 (V_{F_2} - V_{F_1})^{-1},$$

where

$$D = P_2 - P_1;\ h = (F_1 - P_1)\ (D)^{-1}$$

Results and discussion

Grain yield per plant of each inbred line did not significantly differ from low to high population densities. Concerning the F_1 hybrids data from Table 1 showed that grain yield per plant of the 15 single crosses decreased as plant density increased. However, t-test, revealed that five single crosses, i.e. (Sc_6, Sc_7, Sc_8,

Table 1

Means of grain yield/g/plant of parents, F_1, F_2, Bc_1 and Bc_2 populations for 15 single crosses at two plant densities i.e. low/L/ and high/H/

Generation Cross	Density	P_1	P_2	F_1	F_2	Bc_1	Bc_2	A	B	C	t-test
Sc_1	L	69	63	220	149	108	123	10*	—67	—77	ns
	H	76	52	192	152	135	125	36	27	—12	
Sc_2	L	69	67	222	153	159	146	16	29	5	ns
	H	76	69	183	149	177	136	39	102	32	
Sc_3	L	69	64	211	159	170	157	39	66	72	ns
	H	76	65	198	166	157	132	57	52	—9	
Sc_4	L	69	67	160	125	133	128	21	38	54	ns
	H	76	75	175	153	130	127	56	12	7	
Sc_5	L	69	77	187	170	176	146	85	89	66	ns
	H	76	78	201	176	172	132	150	92	23	
Sc_6	L	63	67	198	190	152	139	118	39	30	*
	H	52	69	188	177	156	123	127	66	18	
Sc_7	L	63	64	204	111	116	144	—45	—36	40	**
	H	52	65	172	134	143	116	44	46	4	
Sc_8	L	63	67	184	165	120	106	82	—12	—74	*
	H	52	75	164	152	126	103	89	14	—42	
Sc_9	L	63	77	176	163	155	123	87	58	—2	ns
	H	52	78	175	164	170	112	102	87	—32	
Sc_{10}	L	67	64	182	177	155	142	120	69	83	ns
	H	69	65	186	166	170	120	136	109	13	
Sc_{11}	L	67	67	188	108	126	140	—39	—3	49	ns
	H	69	75	192	152	135	137	44	4	19	
Sc_{12}	L	67	77	209	146	178	141	17	70	4	ns
	H	69	78	184	148	174	126	44	87	—10	
Sc_{13}	L	64	67	196	151	145	112	43	27	—73	*
	H	65	75	168	153	163	106	75	83	—50	
Sc_{14}	L	64	77	203	180	150	159	93	21	92	**
	H	65	78	157	159	155	136	97	76	87	
Sc_{15}	L	67	77	173	129	122	141	18	—6	76	ns
	H	75	78	169	158	161	138	73	75	60	

* Not significant different for A, B, C scaling test.

** Significant at 0.01 level of probability.

Sc_{13} and Sc_{14}) were significantly decreased, while the other ten crosses did not significantly differ by increasing plant density from 20 to 30 thousand plants/fad. These results agree with those reported by Russell and Teich (1967), Geadelmann and Peterson (1978) and Salem et al. (1985). It was found that the cross (Sc_1) gave the highest grain yield per plant, followed by (Sc_2) at the two population densities on the average.

The correlation coefficient between the two plant densities for grain yield was highly significant for single crosses ($r=0.78$**) and all genotypes ($r=0.98$**), while that relationship for inbreds was positive ($r=0.66$), but not significant. These results indicate that the rank of single crosses is more stable than inbreds under stress conditions. This means that the high yielding single crosses, under low population density was still the higher one under high population density. While inbred lines, either high or low density differed with increasing plant density. These results could be discussed according to the findings of Funk and Anderson (1964), Eberhart and Russel (1966), Hallauer (1972) and Galal et al. (1984) who demonstrated that heterogeneous population tended to have better yield stability than homogeneous ones.

Table 1 gives the mean of grain yield performance of parents, F_1, F_2 and back cross generations for 15 single crosses of maize at two plant densities. The F_1 values were higher than the two parental lines for both environments (low and high population density).

These results indicate that the non-additive effects represent the major genetic effect for the inheritance of grain yield in the study of these crosses.

Table 2 shows the mean of grain yield performance of parents, F_1, F_2 and back cross generations for the combined over two environments in the 15 single crosses of maize.

Table 2

Means of grain yield/g/plant of parents, F_1, F_2, Bc_1 and Bc_2 populations for the combined over two plant densities in 15 single crosses of maize

Cross Generation	Sc_1	Sc_2	Sc_3	Sc_4	Sc_5	Sc_6	Sc_7	Sc_8	Sc_9	Sc_{10}	Sc_{11}	Sc_{12}	Sc_{13}	Sc_{14}	Sc_{15}
P_1	72	72	72	72	72	57	57	57	57	68	68	68	64	64	71
P_2	57	68	64	71	77	68	64	71	77	64	71	77	71	77	77
F_1	206	202	205	167	194	193	183	174	176	184	190	196	182	180	171
F_2	150	151	162	139	173	181	122	158	164	172	130	147	152	169	144
Bc_1	121	168	164	132	174	154	129	123	165	162	131	176	154	153	141
Bc_2	124	141	144	127	139	131	130	103	117	131	138	121	109	148	139
A*	22	27	48	38	117	133	5	85	94	128	2	30	59	95	45
B	−20	66	59	25	90	53	12	4	72	89	4	78	55	49	34
C	−45	19	32	30	45	24	32	−64	−16	48	34	−53	−62	89	68
$F_1-MP/MP\%$	291**	189**	201**	134**	160**	176**	202**	172**	163**	179**	173**	138**	170**	155**	131**
$F_1-F_2/F_1\%$	27**	25**	21**	17**	21**	6**	61**	9**	7**	12**	32**	25**	16**	6**	16**

* Not significant different for A, B, C scaling test.
** Significant at 0.01 level of probability.

Table 3

Estimates of additive (D) dominance (H) variances, average degree of dominance ($\bar{a}$) and minimum number of genetic factors for grain yield/plant of 15 maize single crosses in two plant densities and combined

Cross	(D)			(H)			($\bar{a}$)			Sewall-Wright (g. f.)		
	L	H	C	L	H	C	L	H	C	L	H	C
$Kfs_1 \times Kfs_2$	2815*	3364**	2893**	− 628	− 115	− 176	0.668	0.261	0.349	6.62	3.05	5.05
$Kfs_1 \times Kfs_3$	3238*	2897*	6039**	203	333	−1239	0.354	0.479	0.641	4.69	2.09	1.71
$Kfs_1 \times Kfs_4$	3616**	4472**	4724**	453	−1083	−1079	0.158	0.696	0.676	4.32	3.04	3.46
$Kfs_1 \times Kfs_5$	1752	8283**	4528**	327	−3000	−1015	0.611	0.851	0.670	2.99	1.86	1.32
$Kfs_1 \times Kfs_6$	2315	1503	2982**	966	1628*	883	0.913	1.472	0.770	3.31	1.46	1.43
$Kfs_2 \times Kfs_3$	3044*	7742**	2893**	− 158	1320*	74	0.323	1.847	0.226	2.78	4.15	2.45
$Kfs_2 \times Kfs_4$	3319**	0000	1516*	− 573	2854*	740	0.588	0.000	0.988	3.78	8.45	9.80
$Kfs_2 \times Kfs_5$	0000	0000	0000	143	605	289	0.000	0.000	0.000	5.00	4.82	6.15
$Kfs_2 \times Kfs_6$	2554	0000	836	1035*	1557**	1696**	0.900	0.000	2.015	1.07	2.98	2.27
$Kfs_3 \times Kfs_4$	7233**	6758**	7097**	−1355	−1407	1419	0.612	0.645	0.632	0.65	0.69	0.84
$Kfs_3 \times Kfs_5$	4500**	5828**	4901**	−1069	−2977	−1329	0.689	0.844	0.736	1.56	1.53	2.74
$Kfs_3 \times Kfs_6$	2146*	2491*	2951*	177	− 212	− 624	0.406	0.584	0.650	5.89	6.16	6.58
$Kfs_4 \times Kfs_5$	1498	1354	721	1508*	871	1215**	4.645	1.134	1.836	8.84	6.52	5.84
$Kfs_4 \times Kfs_6$	4512**	2960**	5141**	− 305	151	− 797	0.368	0.335	0.557	2.16	1.09	1.43
$Kfs_5 \times Kfs_6$	1924	5510**	3361**	1220*	− 997	506	1.126	0.602	0.549	1.24	0.83	1.01

L = Low density, H = High density, C = Combined
* Significant at 0.05 level of probability.
** Significant at 0.01 level of probability.

The F_1 values as percent of mid-parents ranged from 219% to 131%, which means that the heterosis as percent of mid-parent for all crosses were highly significant. The different between F_2 and F_1 as percentage of inbreeding depression ranged from 6% to 61% and was highly significant for all crosses. It means that the non-additive gene actions played important role in the inheritance of grain yield under the condition of this study. The A, B and C scaling test values as shown in Tables 1 and 2 for grain yield in the 15 single crosses were not significant. Consequently, the simple model of Mather and Jinks (1982) is adequate to explain the inheritance of grain yield in the present study.

Table 3 gives the estimates of additive (D) dominance components, average degree of dominance and minimum number of genetic factors for grain yield per plant at the two plant densities and the combine of them in the 15 crosses of maize. The additive genetic variances were significantly important sources for the genetic variability in most of all crosses studied. While dominance was only significant from zero for one cross at low density and two crosses of the combined. The degree of dominance for most crosses under different environments were less than one, suggesting that they were in the partial dominance range. While in some crosses exceeded than unit and they were in the overdominance range. This suggest that the dominance appears to provide the explanation of heterosis. In general, Gamble (1962) reported that the dominance gene effects were important in the inheritance of yield. El-Demerdash (1972) indicated that the additive genetic variance was equal to dominance variance for grain yield. These results are similar to that reported by Robinson et al. (1955), Gardner and Lonnquist (1959) and Moll et al.

Table 4

Estimates of heritability (h^2) and expected genetic advance ($\Delta g\%$) in broad and narrow senses for grain yield/plant of 15 maize single crosses in two plant densities and combined

Cross	h^2 (broad)			h^2 (narrow)			Δg in h^2 of broad %			Δg in h^2 of narrow %		
	L	H	C	L	H	C	L	H	C	L	H	C
$Kfs_1 \times Kfs_2$	0.78	0.79	0.69	0.61	0.76	0.62	67	72	61	52	70	55
$Kfs_1 \times Kfs_3$	0.74	0.75	0.80	0.69	0.68	0.69	60	64	97	57	57	77
$Kfs_1 \times Kfs_4$	0.74	0.77	0.77	0.73	0.55	0.71	58	89	83	57	68	64
$Kfs_1 \times Kfs_5$	0.66	0.86	0.78	0.56	0.34	0.47	51	93	93	43	46	72
$Kfs_1 \times Kfs_6$	0.73	0.75	0.77	0.51	0.36	0.60	60	54	62	57	40	61
$Kfs_2 \times Kfs_3$	0.70	0.68	0.73	0.63	0.45	0.71	64	56	54	53	48	39
$Kfs_2 \times Kfs_4$	0.68	0.64	0.67	0.61	0.28	0.45	52	38	46	44	33	35
$Kfs_2 \times Kfs_5$	0.55	0.52	0.50	0.48	0.36	0.38	74	53	64	52	36	31
$Kfs_2 \times Kfs_6$	0.76	0.63	0.70	0.54	0.40	0.43	88	98	93	81	64	69
$Kfs_3 \times Kfs_4$	0.86	0.85	0.86	0.62	0.73	0.72	86	81	94	64	71	68
$Kfs_3 \times Kfs_5$	0.79	0.82	0.81	0.64	0.67	0.74	46	59	71	41	54	57
$Kfs_3 \times Kfs_6$	0.68	0.77	0.88	0.63	0.73	0.83	51	66	59	43	40	22
$Kfs_4 \times Kfs_5$	0.65	0.72	0.68	0.55	0.44	0.26	69	63	83	64	60	70
$Kfs_4 \times Kfs_6$	0.80	0.76	0.80	0.76	0.72	0.75	59	64	69	42	31	53
$Kfs_5 \times Kfs_6$	0.78	0.82	0.81	0.48	0.61	0.70	62	78	71	38	74	61

(1964). However, the estimates of additive and dominance genetic variances might be used in the planning of a prospective breeding program using the advanced generation of the 15 crosses as a source of new derivative inbred lines. It was evident from Table 3 also that the minimum number of gene factors were varied for the cross and among the crosses at the different environments, ranging from one to nine pairs approximately for the grain yield per plant of the different crosses in this study.

Table 4 presents the heritability and expected genetic advance in broad and narrow senses for the 15 crosses at the three environments. The heritabilities in broad senses were quite high for grain yield per plant but they were little low in narrow sense heritabilities in all crosses. The heritability in narrow sense values at the three environments ranged from 0.26 to 0.76 and these are expected due to the additive genetic effects in most crosses. It is likely that the environmental deviation plays a serious role and could bias the estimates of genetic variance components. Consequently, the application of selection programme and the hydridization programme for the advanced generation of these crosses would be effective in deriving new inbred lines for improving hybrid production. Nevertheless, the expected gain from selection in broad and narrow senses of heritabilities for different crosses in relation to grain yield appeared that the recurrent selection during the first selfing generation (S_1) would be effective to obtain the best inbred lines for future hybrid programs.

Generally, from the results obtained in the present study, it may be concluded that these 15 single crosses would be a good source for deriving new inbred lines having high general combining ability of grain yield during the advanced generation. Consequently, this would be effective for developing new hybrids having good specific combining ability among their parental lines.

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Plant Cultivation

IMPROVEMENT OF PRODUCTIVITY AND QUALITY OF *LENS CULINARIS* BY PYRIDOXINE AND PHOSPHORUS APPLICATION

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In a factorial randomised field trial, the effect of soaking lentil (*Lens culinaris* L. Medic.) var. T 36 seeds for 12h in 0, 0.2, 0.3 and 0.4% aqueous pyridoxine solution and of 15, 30, 45 and 60 kg P/ha, and of their interaction was studied on nitrate reductase activity (NRA) at 60, 90 and 120 days, net assimilation rate (NAR) at 60—90 and 90—120 days and pods plant^{-1}, length pod^{-1}, seeds pod^{-1}, 1,000 seed weight, seed yield and seed protein content at 140 days (harvest). The application of 0.3% pyridoxine and 30 kg P/ha separately and the interaction between 0.2% pyridoxine × 30 kg P/ha proved optimum for most of the parameters studied, except the interaction effect for 1,000 seed weight. NRA and NAR at all samplings showed a strong correlation ($p < 0.01$) with seed yield and seed protein content, while yield attributes showed a similar correlation with seed yield only. Thus, pre-sowing seed treatment with 0.3% pyridoxine in combination with 30 kg P/ha ensures a high yield and quality of lentil.

Keywords: lentil, nitrate reductase, phosphorus, pyridoxine

Introduction

Leguminous crops by virtue of their high grain protein content, i.e. 20—40%, have been unequivocally proved indispensable in tropical and sub-tropical regions of the world, including India, in particular, where the majority of the population depends mostly upon vegetable proteins. Moreover, they furnish inexpensive and nonpolluting nitrogen to the soil through symbiotic dinitrogen fixation. Ironically, the so-called "Green Revolution", which succeeded in meeting the increasing demand for cereals during the recent past to cope with the "population explosion", had eclipsed the need for increased production of grain legumes. Therefore, neither the genetic stock nor the agronomic practices for the cultivation of leguminous crops could be improved. The ignorant farmers continued to grow low-yielding cultivars of legumes, often as intercrops, in unirrigated areas. This resulted in low yields although they are grown on 24 million hectares of arable land of India, which is the largest area of the world occupied for the cultivation of leguminous crops (Mehta, 1968). Hence, it is not surprising to note that the total production of grain legumes in the country is only about 12 million tonnes, i. e. 500 kg/ha (Jeswani and Van Schaik, 1968; Mann and Singh, 1975) as against 3,494 kg/ha produced by

France, for example (Anonymous, 1984). The situation prompted national planners to give top priority to improving the genetic form of these crops and to work out the set of conditions for full exploitation of their potential.

At Aligarh also, a modest attempt has been made to improve the productivity and quality of some newly evolved grain legumes by judicious application of nutrients and vitamins, particularly B_6 (pyridoxine), as a substantial yield increment due to its exogenous application has been achieved in mungbean by Samiullah et al. (1985). The stimulation of root growth in many crops, including mungbean and lentil, by administering a dilute aqueous solution of pyridoxine to seeds (Oertli, 1987; Afridi et al., 1979; Samiullah et al., 1985; Khan and Ansari, 1984) led us to assume that this beneficial effect would favour an ever greater uptake of nutrients by enhancing the area of contact between roots and soils, even in the case of limited availability. This has an added advantage for soil-applied phosphorus, which is very prone to be fixed in the soil (Russell, 1950). Keeping these considerations in view, a field experiment was undertaken to investigate the effect of pre-sowing seed soaking with aqueous pyridoxine solution separetely or in combination with various doses of phosphorus on the performance of lentil.

Materials and methods

The experiment was conducted for one year during the 'rabi' season at the Agricultural Farm of Aligarh Muslim University, Aligarh. The soil was sandy loam (pH: 7.8; available N 219 kg/ha; available P 18 kg/ha and available K 668 kg/ha). Seeds of lentil (*Lens culinaris* L. Medic.) soaked for 12h in 0, 0.2, 0.3 and 0.4% aqueous pyridoxine solution were inoculated with rhizobium and sown in a 25 m^2 plots at the rate of 50 kg/ha. Four doses of phosphorus (15, 30, 45 and 60 kg P/ha) were applied to the soil in sixteen possible combinations with the pyridoxine soaking treatment according to a factorial randomized block design. A recommended uniform basal dose of 45 kg N and 30 kg K/ha was also applied. Each treatment was replicated three times. The sources of nitrogen, phosphorus and potassium were urea, monocalcium, super-phosphate and muriate of potash respectively. Leaf NRA was assayed at 60, 90 and 120 days after sowing according to Jaworski (1971), and NAR at 60—90 and 90—120 days was computed according to the method described by Milthorpe and Moorby (1979). On the other hand, various yield attributes, including pod number $plant^{-1}$, length pod^{-1}, seed number pod^{-1} and 1,000 seed weight, and seed yield and protein content as described by Lowry et al. (1951) were studied at 140 days after sowing, i.e. at harvest.

Results and discussion

Effect of pyridoxine treatment

Among the soaking treatments, 0.3% aqueous pyridoxine solution increased leaf NRA by 23.3% at 60 days, 42.7% at 90 days and 87.4% at 120 days compared with the water soaked control (Table 1). The stimulation of NRA by pyridoxine has been well established in summer moon by Samiullah et al. (1985). The assumed reason for such stimulation is that pyridoxine, being a root growth factor (Bonner and Bonner, 1948), promoted the uptake of nutrients including NO_3^-, which induced and stabilised the nitrate reductase in the leaf (Hewitt and Afridi, 1959; Afridi and Hewitt, 1962).

Table 1

Effect of pre-sowing seed treatment with pyridoxine and phosphorus application on leaf nitrate reductase activity (n mol NO_2^-/g/h) of lentil var. T—36 (Mean of three replicates)

Phosphorus kg P/ha	% aqueous pyridoxine solution														
	0	0.2	0.3	0.4	Mean	0	0.2	0.3	0.4	Mean	0	0.2	0.3	0.4	Mean
	60 days ↑					90 days ↑					120 days ↑				
15	107.84	118.24	124.37	111.53	115.50	79.57	96.65	107.68	83.95	91.91	59.07	45.77	77.45	45.34	56.91
30	116.93	146.02	139.57	121.57	131.02	87.44	118.97	113.44	101.40	105.31	54.42	98.43	101.44	64.23	79.63
45	107.94	133.38	146.32	122.90	127.64	75.50	113.86	119.34	103.86	103.17	43.33	87.01	105.80	73.54	77.42
60	103.36	127.89	127.64	122.45	120.34	73.66	107.56	110.67	103.33	98.81	41.86	81.33	87.76	68.63	69.89
Mean	109.02	121.38	134.48	119.61		78.99	109.26	112.78	98.16		49.67	78.14	93.11	62.94	

	C. D. at 5% at 60 days	C. D. at 5% at 90 days	C. D. at 5% at 120 days
Pyridoxine	1.34	0.31	1.66
Phosphorus	1.34	0.31	1.66
Pyridoxine × Phosphorus	2.68	0.62	3.32

Table 2

Effect of pre-sowing seed treatment with pyridoxine and phosphorus application on net assimilation rate ($g/m^2/d$) of lentil var. T—36 (Mean of three replicates)

Phosphorus kg P/ha	% aqueous pyridoxine solution									
	0	0.2	0.3	0.4	Mean	0	0.2	0.3	0.4	Mean
	60—90 days					90—120 days				
15	3.236	3.939	4.221	3.651	3.761	1.877	1.983	2.129	1.947	1.984
30	3.729	5.168	5.129	3.978	4.501	1.949	2.441	2.330	2.007	2.182
45	2.969	4.784	5.741	4.187	4.420	1.370	2.305	2.460	2.101	2.059
60	2.722	4.386	4.685	3.985	3.945	1.215	2.140	2.286	2.052	1.923
Mean	3.164	4.569	4.944	3.950		1.603	2.217	2.301	2.027	

	C. D. at 5% at 60—90 days	C. D. at 5% at 90—120 days
Pyridoxine	0.29	0.012
Phosphorus	0.29	0.012
Pyridoxine × Phosphorus	0.58	0.024

The same treatment also enhanced the NAR at both dates. The increase over the control due to 0.3% pyridoxine treatment was 56.2% at 60—90 days and 43.5% at 90—120 days (Table 2). This indicates that plants receiving pyridoxine as soaking treatment had enhanced photosynthetic activity as a result of growth promotion of above and under-ground plant parts. This beneficial effect has been ultimately manifested in the yield performance of the crop that produced increases of 70.6%, 24.0%, 72.9%, 6.4%, 29.1% and 12.8% in pod number plant^{-1}, length pod^{-1}, seed number pod^{-1}, 1,000 seed weight, seed yield and seed protein content, respectively, in 0.3% pyridoxine treatment more than in the water soaked control (Tables 3—4). This suggests that pyridoxine treatment not only enhanced the assimilatory ability of the crop but also maintained a balance in the source-sink relationship. This action of pyridoxine has been well documented in field-grown cereals and leguminous crops (Ahmad et al., 1981; Samiullah et al., 1985).

Effect of phosphorus treatment

With regard to the phosphorus application, 30 kg P/ha registered a significant maximum in NRA at 60, 90 and 120 days, NAR at 60—90 and 90—120 days, pod number plant^{-1}, pod length, seed number pod^{-1}, 1,000 seed weight, and seed yield and seed protein content at harvest. The application of phosphorus at the rate of 30 kg/ha increased NRA by 13.4%, 14.5% and 39.9% at 60, 90 and 120 days; NAR by 19.6% and 9.9% at 60—90 and 90—120 days; pod number plant^{-1} by 28.0%, length pod^{-1} by 9.0%, seed number pod^{-1} by 23.1%, 1,000 seed weight by 2.8%, seed yield by 10.2% and seed protein content by 7.1% over the control, i.e. 15 kg P/ha (Tables 1—4). A similar effect of phosphorus application on lentil performance has been reviewed by Saxena (1981) and is suggested to be a manifestation of the improved growth and proliferation of roots (Chowdhury et al., 1974; Sekhon et al., 1983).

Table 3

Effect of pre-sowing seed treatment with pyridoxine and phosphorus application on yield parameters of lentil var. T—36 (Mean of three replicates)

Phosphorus kg P/ha	% aqueous pyridoxine solution														
	0	0.2	0.3	0.4	Mean	0	0.2	0.3	0.4	Mean	0	0.2	0.3	0.4	Mean
	Pod number plant^{-1}					Length pod^{-1}					Seed number pod^{-1}				
15	70.24	80.14	100.24	72.33	80.74	0.790	0.820	0.900	0.800	0.828	1.03	1.62	1.80	1.08	1.38
30	78.93	137.33	112.66	84.53	103.36	0.810	1.020	0.950	0.830	0.903	1.25	1.92	1.87	1.75	1.70
45	61.66	107.88	144.64	92.55	101.68	0.770	0.930	1.050	0.880	0.908	1.00	1.85	1.93	1.79	1.64
60	60.44	102.21	105.33	92.33	90.08	0.710	0.900	0.920	0.871	0.850	0.99	1.81	1.81	1.77	1.60
Mean	67.82	106.89	115.72	85.44		0.770	0.918	0.955	0.845		1.07	1.80	1.85	1.60	

	C. D. at 5% for pod number plant^{-1}	C. D. at 5% for length pod^{-1}	C. D. at 5% for seed number pod^{-1}
Pyridoxine	3.67	0.016	0.023
Phosphorus	3.67	0.016	0.023
Pyridoxine × Phosphorus	7.34	0.031	0.047

Table 4

Effect of pre-sowing seed treatment with pyridoxine and phosphorus application on yield parameters and seed protein content of lentil var. T—36 (Mean of three replicates)

Phosphorus kg P/ha	% aqueous pyridoxine solution														
	0	0.2	0.3	0.4	Mean	0	0.2	0.3	0.4	Mean	0	0.2	0.3	0.4	Mean
	1000 seed weight (g)					Seed yield (q ha^{-1})					Protein content (%)				
15	19.93	20.61	21.08	20.00	20.41	18.10	19.86	21.49	19.62	19.77	20.80	20.00	21.50	20.40	20.68
30	20.53	21.30	21.28	20.88	21.00	19.74	23.72	23.61	20.09	21.79	20.20	24.60	23.60	20.20	22.15
45	19.88	21.23	21.36	20.98	20.86	17.82	23.50	24.65	21.41	21.85	20.00	22.80	24.20	20.80	21.95
60	19.48	21.15	21.19	20.96	20.70	16.46	22.88	23.33	21.29	20.99	19.80	21.40	21.80	20.60	20.90
Mean	19.96	21.07	21.23	20.71		18.03	22.49	23.27	20.60		20.20	22.20	22.78	20.50	

	C. D. at 5% for 1.000 seed weight	C. D. at 5% for seed yield	C. D. at 5% for protein content
Pyridoxine	0.392	0.47	0.52
Phosphorus	0.392	0.47	0.52
Pyridoxine × Phosphorus	N. S.	0.93	1.04

N. S. = Non significant

Effect of interaction of pyridoxine and phosphorus treatment

The interaction between pyridoxine soaking and basal phosphorus application was found significant for all characteristics studied and enhanced all parameters compared to those achieved (Tables 1—4). Among all interactions, it was concluded that soaking seed in 0.2% pyridoxine solution in combination with 30 kg P/ha proved optimum except for 1,000 seed weight, and increased NRA by 35.4% at 60

Table 5

Correlation of various parameters with seed yield and seed protein content of lentil var, T—36 (number of observation, n=48)

Parameters	Days	Correlation coefficient (r)	
		Yield	Protein
Nitrate reductase activity	60	0.649**	0.567**
	90	0.608**	0.510**
	120	0.548**	0.468**
Net assimilation rate	60— 90	0.635**	0.546**
	90—120	0.521**	0.492**
Pod number plant^{-1}	140	0.705**	—
Length pod^{-1}	140	0.316*	—
Seed number pod^{-1}	140	0.398**	—
1.000 seed weight	140	N. S.	—

* Significant at 5%; ** Significant at 1% and N. S. = Non significant

days, 49.8% at 90 days and 79.1% at 120 days, NAR by 59.7% at 60—90 days and 30.1% at 90—120 days, pod number plant^{-1} by 95.5%, length pod^{-1} by 29.1%, seed number pod^{-1} by 86.4%, seed yield by 31.1% and seed protein content by 18.2% over their respective controls (water-soaked seed × 15 kg P/ha). Thus, soaking treatment with pyridoxine and basally applied phosphorus, which is responsible primarily for root growth and proliferation, as discussed above, seems to have acted synergistically, since the percentage increase was greater for the different attributes after combined application than the sum of the individual effects.

It is inferred from Table 5 that NRA and NAR at all samplings showed a strong correlation ($p<0.01$) with seed yield and seed protein content, while yield attributes showed a similar correlation with seed yield only. This depicts the dependence of seed yield and protein content upon the complete vegetative and reproductive performance of the crop.

Conclusively, augmented seed yield and quality may be obtained in lentil if the plants receive 0.3% pyridoxine as pre-sowing seed treatment and 30 kg P/ha as basal dressing. Moreover, the soaking treatment can be combined with the routine practice of seed inoculation with rhizobium, and leads to no economical burden upon farmers.

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CRITICAL DEFICIENCY LEVEL AND FIELD RESPONSE OF RICE TO Zn APPLICATION ON TYPIC USTOCHREPTS

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Field trails were conducted at 20 locations representing typic Ustochrepts having a wide range in DTPA extractable Zn to determine the critical deficiency level of Zn for predicting response of rice to Zn application. Soil application of 5.6 kg Zn ha^{-1} significantly increased the grain yield in deficient soils, emphasizing the need for Zn fertilization of rice grown on these soils. Soil Zn extracted by DTPA was significantly related with percent grain yield increase over zero Zn ($r = -0.78^{**}$). A critical level of 0.82 mg Zn kg^{-1} soil extractable by DTPA separated the Zn deficient soils from the sufficient ones. This level gave a predictability value of 90%.

Keywords: coarse textured soils, critical level, response of Zn, rice

Introduction

Rice is an important crop in the Indian subcontinent. The poor yield of this crop despite adequate application of NPK fertilizers in many parts of India has been ascribed to the inadequate supply of Zn from the soil. Its deficiency in rice is so widespread that it ranks next in its importance to nitrogen. The response of rice to the application of Zn in different rice growing countries of the world has been reported (Katyal and Ponnamperuma, 1974; Takkar and Nayyar, 1986). The need to add Zn fertilizers to soils depend upon their available Zn status. Therefore, the establishment of a critical level of Zn in soils is a prerequisite to separate Zn deficient from sufficient soils. The adequate levels of Zn vary from soil to soil and crop to crop. However, information is lacking regarding critical level of soil-available Zn for predicting profitable response to Zn application for rice culture on typic Ustochrepts soils of Punjab, where the crop is widely grown in rotation with wheat. Also the DTPA method of Lindsay and Norvell (1978) has been found to be most suitable and efficient in predicting response of rice to applied Zn (Ponnamperuma et al., 1981, Singh and Takkar, 1981). This extractant was therefore employed for determining the available Zn status in soils. The present investigation was undertaken to establish the critical level of DTPA extractable Zn in these soils for rice and to examine the extent of response of crop to the application of Zn based on initial Zn status.

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Materials and methods

Field trials were conducted at 20 locations on typic Ustochrepts with a range in diethylene triamine penta acetic acid (DTPA) extractable Zn, in the Sangrur district of the Punjab State, India. The area lies between latitudes 29°56′13″ and 30°31′30″ N and longitudes 75°33′ and 76°19′ E. Forty-five-day-old seedlings of long-duration rice variety PR 106 were transplanted and grown during kharif 1988. The experiment was a randomized block design with a plot area of 50 sq.m for each treatment. All the plots received a basal dose of N, P and K at 60, 13 and 25 kg ha^{-1} as urea, superphosphate and muriate of potash, respectively at transplanting before the last puddling. A further 60 kg N ha^{-1} was applied as urea in two equal splits at an interval of 3 weeks after transplanting.

The fertilizer treatments consisted of (i) zero Zn (ii) 5.6 kg Zn ha^{-1} as $ZnSO_4$. Zinc in the soil was applied by broadcast at the time of transplanting the rice seedlings. The crops were transplanted in the 2nd week of June, 1988 and were harvested in the 2nd week of Oct. 1988. From each experimental site, representative soil samples were collected before fertilization and sowing of the crop. Soil samples were taken with an augar so as to get a uniform core from the surface to the plough depth (0—15 cm) from 6—8 spots at random from each site. The samples were air dried in the shade, well mixed, and a composite sample of about 0.5 kg was taken. The soil samples were analysed for texture, pH, organic carbon and available P by standard procedure (Page, 1982). The samples were analysed for their initial available Zn content by extraction with a DTPA buffer solution (diethylene triamine penta acetic acid containing 0.1 M triethanol amine and 0.01 M $CaCl_2$ adjusted to pH 7.3 with distilled H_2O) using a soil: solution ratio of 1 : 2 and a shaking time of 2 hours (Lindsay and Norvell, 1978). Zinc in the filtrates was determined by atomic absorption spectrophotometry.

The crop was grown to maturity and the grain yield of the control, as well as of the Zn treatments, were recorded. The critical deficiency level of Zn in soil was determined by the statistical model of Cate and Nelson (1971).

Results and discussion

Soil characteristics and available Zn extracted by DTPA extraction are given in Table 1. Soil texture varied from loamy sand to loam, pH from 8.2 to 9.3, organic carbon from 0.33 to 0.79%, and available P from 10 to 75 kg ha^{-1}. DTPA extractable Zn varied from 0.30 to 3.60 mg kg^{-1} soil with a mean value of 1.42.

Critical level of Zn

The grain yield of rice in the control plots increased as the DTPA extractable Zn in soil increased, apparently as a results of the greater availability of Zn to the plants. Also percent grain response to applied Zn decreased as the DTPA extractable Zn increased ($r = 0.78^{**}$). This shows that the DTPA Zn can successfully be used to monitor the Zn availability to rice in the investigated soils studied. The critical level of available Zn was obtained by plotting yield increase over zero Zn against DTPA extractable Zn values, in accordance with the graphical method proposed by Cate and Nelson (1965). This value was also calculated by using the statistical model of Cate and Nelson (1971). Both the approaches gave the same critical value of 0.82 mg Zn kg^{-1} soil for predicting the response of rice to Zn (Fig. 1). The probability of the response of rice to Zn application in soil testing, less than this critical level of 0.82 mg Zn kg^{-1} soil, was 89%, and in soils testing greater than 0.82 mg Zn kg^{-1} soil was only 9%. It suggests that a critical level of 0.82 mg Zn kg^{-1} soil can safely be used for demarcating the deficient soils from sufficient ones for rice crop.

Critical deficiency levels of Zn have been reported to differ markedly in soils for the same crop. It varied from 0.45 to 2.0 mg Zn kg^{-1} soil in red and black soils which was considerably higher than the range of 0.38 to 0.90 mg Zn kg^{-1} soil in alluvial soils for rice (Takkar and Nayyar, 1986). The critical value of available Zn in Phillipines soils for rice has been reported to be 1.0 mg Zn kg^{-1} soil extractable by 0.01 M HCl (Katyal and Ponnamperuma, 1974) and 0.83 mg for DTPA extractable Zn kg^{-1} soil (Ponnamperuma et al., 1981). The critical level of 0.82 mg Zn kg^{-1} soil established under field conditions in the present study is very close to the value of 0.86 mg Zn kg^{-1} soil reported under greenhouse conditions (Singh and Takkar, 1981).

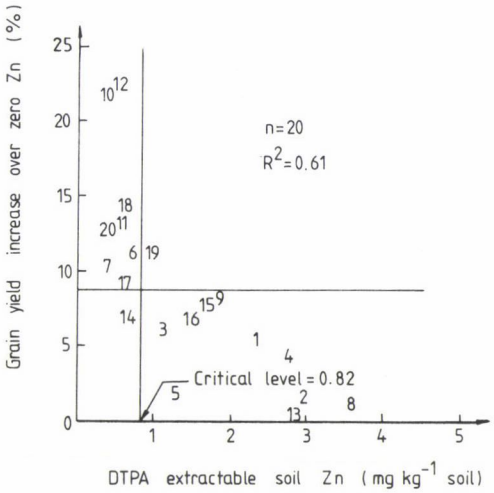

Fig. 1. Scatter diagram of percent yield increase over ZN in rive verses DTPA — Zn in various soils. Numbers indicate the soils as shown in Table 1

Response of rice to Zn

The rice grain yield significantly increased by the application of 5.6 kg Zn ha^{-1} to the experimental soils testing less than 0.82 mg Zn kg^{-1} soil. Above this level, responses were non-significant. The grain yield in the absence of applied Zn ranged from 5.38 to 8.08 t ha^{-1} as compared to 5.75 to 8.33 t ha^{-1} in Zn treated soils (Table 1). The mean grain yield on Zn deficient soils was 6.59 t ha^{-1} and it increased to 7.5 t ha^{-1} in Zn treated soils. Similarly, on Zn sufficient soils, the mean yield in control treatment was 6.85 t ha^{-1}, and in the Zn treated soils it was 7.2 t ha^{-1}. The increase in yield in soils deficient in Zn was 0.91+0.35 t ha^{-1}, while the increase in Zn sufficient soils was 0.35+0.22 t ha^{-1}. Of the 9 soils containig less than 0.82 mg Zn kg^{-1} soil, eight soils responded to Zn application (Table 2). The mean percent increase in yield in the Zn deficient was 13.7 whereas in Zn sufficient soils it was only 5.3%. Similarly, on Zn sufficient soils out of 11 only one soil responded to Zn application.

Table 1

Some characteristics of the soils and grain yield of rice at different locations

Soil	DTPA Zn (mg kg^{-1}) soil	Texture	pH (1 : 2) Soil: water suspension	Organic carbon (%)	P (kg ha^{-1})	Grain yield (t ha^{-1})	
						Control	5.6 kg Zn ha^{-1}
1	2.30	LS*	8.7	0.37	10	6.82	7.22
2	3.00	LS*	8.3	0.41	60	6.79	6.93
3	1.10	LS*	8.3	0.61	60	7.71	8.20
4	2.70	LS*	8.5	0.44	32	6.65	6.97
5	1.30	LS*	8.2	0.45	60	8.08	8.24
6	0.80	LS*	8.2	0.43	69	6.80	7.57
7	0.38	LS*	8.6	0.37	32	6.78	7.48
8	3.60	SL	8.4	0.79	74	7.65	7.76
9	1.90	LS	8.2	0.49	15	7.48	8.11
10	0.30	LS	8.6	0.34	66	6.23	7.61
11	0.68	LS	8.4	0.64	75	7.37	8.33
12	0.46	LS	8.4	0.43	63	6.63	8.13
13	2.90	LS	8.3	0.46	51	6.76	6.80
14	0.80	SL	8.5	0.40	17	5.38	5.75
15	1.70	L	8.7	0.37	19	5.76	6.25
16	1.60	LS	8.3	0.35	40	5.68	6.09
17	0.72	LS	8.3	0.50	46	7.32	8.03
18	0.64	LS	9.3	0.44	46	6.12	7.01
19	0.84	LS	9.1	0.34	61	6.00	6.69
20	0.50	LS	9.0	0.33	41	6.69	7.57
Mean	1.42		8.5	0.45	47	6.73	7.33
$LSD_{5\%}$	1.00		0.3	0.11	20	7.2	7.6

* SL = sandy loam, L = loam. LS = loamy sand

Table 2

Effect of Zn application on the grain yield of rice. Results are mean values of all soil treatments

Zn status of soils	DTPA Zn (mg kg^{-1} soil)	No. of soils	Grain yield, t ha^{-1}		$LSD_{5\%}$	% yield increased	% soil responding to Zn
			0	5.6 kg Zn ha^{-1}			
Deficient	≤0.82	9	(6.59)* 5.38—7.37	(7.50) 5.75—8.33	(8.6) —	(13.7)	89
Sufficient	>0.82	11	(6.85) 5.68—8.08	(7.20) 6.09—8.24	(ns) —	(5.3)	9

* Figures in parentheses are the mean values

The response of rice to the application of Zn has been reported by several workers (Katyal and Sharma, 1979; Takkar and Nayyar, 1986). Chhibba et al. (1989) reported that 5.0 kg Zn ha^{-1} rate was the optimum for rice grown on calcareous alkaline soils, whereas Bansal and Nayyar (1989) found 11.2 kg Zn ha^{-1} as the optimum level for obtaining a higher yield of rice grown on coarse-textured severely Zn deficient soils. The magnitude of response to applied Zn varied

with soil type and the deficiency status of the soils (Takkar and Nayyar, 1986). In the present study, an average response of 0.91 t ha^{-1} was observed with the application of 5.6 kg Zn ha^{-1} applied through zinc sulphate fertilizer containing 21.0% Zn. These results indicate that a level of 0.82 mg kg^{-1} soil of DTPA extractable Zn separated the deficient soils from the sufficient ones, and emphasizes the need of applying Zn fertilizer on the basis of the soil test, for rice in these soils.

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STUDIES ON THE CULTIVATION METHODS OF *AGASTACHE FOENICULUM* (PURSCH) KUNTZE IN FINLAND*

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Between 1986 and 1990 detailed cultivation experiments were carried out with the perennial anise hyssop (*Agastache foeniculum* (Pursch) Kuntze) plant of North-American origin.

Under the conditions of South-Finland, the vegetation period of the plant grown in direct sowing or seedling raising system is 95—110 days. The average germinative ability of the seed is 78%. In the case of drug production, overwintering is uncertain. Its production is 100—200 kg fresh and 10—25 kg dry, marketable crumbled drug per 100 m². The plant can be successfully grown with chemical-free cultivation methods, with black polythene mulching or on ridges.

Keywords: *Agastache foeniculum* (Pursch) Kuntze, anise hyssop, cultivation trials, drug production

Introduction

Finland is a country in one of the northernmost regions of the Earth, carrying on an extensive agricultural activity. The overproduction of the traditional agricultural produces has made it necessary to seek out and produce new alternative crops, various spice plants, among others. The short and cool vegetation period of the northern regions demands, however, special agricultural methods; or, when choosing the species, preference should be given to the cold-tolerant ones. The introduction of a new plant species not cultivated in a given country so far requires detailed and exact agrobiological studies. This paper sums up the experiences and results obtained in acclimatizing *Agastache foeniculum,* a new spice plant species in Finland.

Origin, botanical description, utilization

According to a review by Lint and Epling (1945) some 20 species of the *Agastache* genus of the family *Lamiaceae (Labiatae)* can be found on the North-American continent from Central Mexico to Canada, and one species *(Agastache rugosa)* is a native of East-China, Manchuria and Japan. The area of the species *Agastache foeniculum* includes the northern states of the USA (Wisconsin, Min-

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nesota, Iowa, Dakota, Colorado) and the southern provinces of Canada (Ontario, Alberta).

The plant was earlier given several synonyms: *Hyssopus anethiodorus* Nutt. 1813, *Hyssopus anisatus* Nutt. 1818, *Hyssopus foeniculum* Spreng. 1819, *Lophantus anisatus* (Nutt) Benth 1829, *Agastache anethiodora* Britton 1898. Its English names are anise hyssop, Blue giant hyssop, fennel giant hyssop and fragrant giant hyssop.

The *Agastache foeniculum* is a 0.8—1 m high perennial plant with smooth, square stalk branching off in the middle. The fibrous root-system is 15—25 cm long, while the leaf is 3—10 cm long, 2—5 cm broad, petiolated, inverse cordate and dentate. The inflorescence is a bluish-lilac verticillate spike, flowering from June to August. Owing to their volatile oil content, recalling the taste and odour of anise, the leaf and flower of the plant have been used to season sweets and desserts, and as a raw material of teas. Due to its decorative appearance it has been planted at the edge of perennial flower beds. In the Soviet Union, in Moldavia, the plant has been examined and cultivated for the purpose of volatile oil production (Arinstein and Rabcsenko, 1978; Kapelev, 1982). According to literary data (Barrett, 1966; Widrechner, 1987) it is a good honey-producing plant.

Although the anise hyssop has long been present in the botanical gardens of Finland, it gained practical importance as a spice plant only in 1981 when a spice plant growing farm began to use and trade it in various tea mixtures. The anise hyssop soon became known and popular in Finland. Besides the tea mixtures, it is used in cakes, ices and sweets; it is particularly favoured in home kitchens. Its flower is a fine and edible decoration for fancy-cakes.

By reason of its aroma, decorativeness, and its short vegetation period suitable to the climatic conditions of Finland, we carried out extensive agrobiological investigations between 1986 and 1990 with the purpose of acquiring an exact knowledge concerning the cultivation possibilities of the plant.

Materials and methods

The cultivation experiments were carried out in Puumala, South-Finland (61° 40′ N, 28° 15′ E), on the trial grounds for medicinal plants of the Helsinki University. The soil of the experiment is a gritty moraine; its characteristics according to the analysis of the Central Laboratory of the Agricultural Research Institute are: pH = 6.4, Ca = 1333, K = 53, Mg = 156, P = 9.5, $N(NH_4) = 4.72$, $N(NO_3) = 4.61$ mg/l. The plots were annually given 500 kg/ha mixed fertilizer (NPK = 7—5—15, Puutarha Y-lannos 2, Kemira Oy) at the beginning of June before planting, and 30 kg/ha N top-dressing (Kalkisalpietari, N = 15%, Kemira Oy) 3 weeks after planting. In the biological cultivation experiments 25 kg/100 m^2 dried poultry granulate (Biolan, Biolan Oy) was applied.

The temperature and precipitation data of the experiments' years are contained in Tables 1 and 2. The initial seed was purchased in 1985 from Canada (Richters, Ontario, Goodwood), and in the subsequent years seed of own production was used.

The seedlings were raised in plastic cells of 5 × 5 cm; 1—3 seeds were sown in each cell. The seedling raising medium was a commercial peat (Vapo). The seedlings were planted in soil covered with black polythene (9/m^2), in potato ridge (6—10—16/m^2) and in uncovered smooth soil (6/m^2). Harvesting was carried out in full blossom; plant height and plant weight, dry matter content, stalk to leaf ratio, fresh and dry crop were measured. The plants cut off at a height of 10—15 cm were dried at 40 °C (10—24 hours), then the leaf and flower parts were removed by crumbling. The volatile oil content and composition of the mixture thus produced were determined in the research laboratory of the Finnish State Enterprise of Alcohol Trade (ALKO Ltd) (Nykäneen et al., 1989).

The seed crop of plants that were not cut off for drug was cleaned, then germination experiments were carried out in 4 replications, with 50 seeds per each sown in Petri dishes of 9 cm in diameter, kept at room temperature (20—24 °C by day and 17—20 °C by night), using the top-paper method.

Having harvested the drug and seed crop, in the spring of the next year, around the end of May — beginning of June, we determined the extent of frost damage by assessing 4 × 20 plants. The mathematical evaluation of the experiments was performed by variance analysis (Sváb, 1981).

Table 1

Average monthly mean temperatures and precipitation sums of summer months in the years of the experiment
(Anon. 1987—1988—1989)

	Year	V	VI	VII	VIII	IX	V—IX
Monthly mean temperature, °C	1930—1960	8.6	13.9	16.7	14.6	9.4	12.6
	1986	10.3	17.3	17.7	13.0	6.3	12.9
	1987	7.8	13.3	15.0	11.2	8.1	9.4
	1988	11.3	16.9	19.7	13.8	11.7	14.7
Monthly precipitation sum, mm	1930—1960	40.0	57.0	69.0	73.0	61.0	300.0
	1986	35.8	23.5	67.6	91.2	73.3	289.4
	1987	29.4	105.9	66.9	122.7	103.6	431.0
	1988	17.3	60.3	36.9	106.7	86.0	307.2

Table 2

Average and minimum temperatures in the winter months of the experimental years, and thickness of the snow-cover
(Anon. 1987—1988—1989)

	Year	X	XI	XII	I	II	III	IV
Temperature °C	1986/87							
	average	4.3	2.1	−11.1	−20.5	− 9.1	− 6.1	1.8
	minimum	−4.0	−4.7	−24.2	−36.2	−22.8	−22.1	−10.4
	1987/88							
	average	5.6	−3.4	− 8.4	− 6.5	− 6.8	− 3.5	0.4
	minimum	−2.0	−16.3	−23.8	−20.3	−22.7	−15.8	−11.6
Snow-cover, cm	1986/87	—	—	32	42	52	47	—
	1987/88	—	23	54	49	72	70	28

Results and evaluation

Germination biology

The anise hyssop seed is small, its thousand-seed-weight — 0.400 g — is relatively constant. The fully mature seeds are deep brown and the less mature ones yellowish-brown. Under favourable weather conditions in long autumns, most seeds become mature in Finland and germinate well. In the upper third or quarter of the spike the seeds do not always become mature. They have a smaller thousand-seed-

Table 3

Results of germination experiments with the seed of anise hyssop
(Puumala, 1986—1989)

No. Origin of seed, cultivation method	Time of harvesting	Beginning of germination	Germination %			Total	1000-seed-weight, g
			7th	14th	21st day		
1. Canada, purchased in 1986	1985	15. III. 1987	88	90	94	94	—
2. Puumala, overwintered, 2-year	6. X. 1987	20. II. 1988	56	61	62	62	—
3. Puumala, overwintered, 2-year	1. X. 1988	16. II. 1989					
a) mixed seed			83	86	89	89	—
b) brown heavy seed			87	90	—	90	0.400
c) yellow light seed			19	25	—	25	0.175
4. Puumala, field, 1st year, main flowering	28. X. 1989	28. XI. 1989	45	80	82	82	0.400
5. Puumala, field, 1st year, sec. fl.	28. X. 1989	28. XI. 1989	40	63	66	66	0.450
6. Puumala, plastic tent, 1st year second flower	28. X. 1989	28. XI. 1989	20	39	42	42	0.420
		Average: (2+3/a+4)				78	0.400

weight (0.175 g) and a much lower germinative ability (25%, Table 3). Seeds of poorer quality can be separated from the mature seeds by a fanner.

According to the data of Table 3, the full germination of the anise hyssop seeds takes 21 days and in the first seven days 85—90% of the germs appear. The seeds retain their germinative ability for 2—4 years. The 62% germinative ability seeds harvested in Puumala in 1987 germinated after 30 months in 9%. The seeds purchased from Canada in 1986 (supposedly harvested in 1985) showed in 1987 94%, in April 1990 — i. e. nearly 5 years later — 21% germinative ability. The average germinative ability of anise hyssop seeds produced in the field in Puumala was 78% between 1987 and 1989. In years with favourable weather conditions, the rate of germination even reached 87—90%; while in the cool, rainy year of 1987 it was lower (62%). After the first drug cutting harvested in July, the second growth also flowered and developed seed capable of germination, both under field (66%) and under plastic house (42%) conditions. Seed production in the field can be mechanized. After mechanical harvesting spontaneous plant emergence was observed for several years.

Length of vegetation, development, overwintering

According to the data of propagation experiments the development of the anise hyssop under conditions of seedling raising took 103 (95—113) days from sowing to full blossoming, i. e. to the first harvest (Table 4). The length of the seedling raising period is 5 weeks and from planting to full blossoming 9—10 weeks generally pass. The plants planted out at the beginning of June reach full height in 2 months and at the time of harvesting a plant weight 250—300 g (Table 5). Under the conditions of Finland, the plants usually reach a height of 70—90 cm and, as a result of intensive fertilization, 120—130 cm high plants have even been found.

Table 4

Length of vegetation of anise hyssop in Puumala in various agrotechnical experiments
(1987—1989)

Sowing time in plastic tent	Sowing-planting day	Planting-harvesting day	Sowing-harvesting day
1987 4 May	42	71	113
10 May	45	62	107
1988 4 May	35	66	101
15 May	26	69	95
28 May	26	74	100
1989 26 April	40	60	100
26 April	40	44*	84*
22 April**	—	—	105

* cultivation in plastic tent, on bench, in pots
** direct sowing in the field

The plants show medium tillering; in our experiments a spacing of 35—40 × 40 cm (6—8 plants per m^2) proved sufficient.

In Finland the anise hyssop can be grown by direct sowing; in 1988, the warmest of the experiment's years, larger leaf yield was attained with irrigated direct sowing than with seedling raising (Table 6). The larger yield is due to the higher plant number (70—80 seeds per running metre). According to our experiences gained with other direct sown spice plant species, the average cool Finnish spring weather delays the germination and reduces the yield. For this reason as well as because of the essentially more manual labour requirement of weed control, cultivation with seedling raising is more expedient and more reliable in Finland.

The overwintering of the plant depends on the time of harvesting and on the weather conditions in late winter. Plants left for seed production overwinter safely, nearly 100%. After the mid-July harvest of leaf and inflorescence, the overwinter-

Table 5

Height, plant weight and yield of anise hyssop at various times of harvesting
(Puumala, 1988—1989)

Harvesting time		Development phases	Plant height, cm		Plant weight, g		Fresh crop, kg/m^2 Density: 6 plants/m^2	
1988	1989	1988	1988	1989	1988	1989	1988	1989
1. 06.7.	18.7.	4—6 foliage leaf	28	51	18	116	0.10	0.69
2. 13.7.	30.7.	beg. of budding	32	54	39	183	0.23	1.10
3. 25.7.	08.8.	beg. of flowering	59	62	103	212	0.62	1.27
4. 02.8.	15.8.	full blossoming	61	63	154	267	0.92	1.60
5. 13.8.	24.8.	end of full bloss	68	67	290	318	1.74	1.91

Planting: 15 June, 1988
15 June, 1989
Spacing: 40 × 40 cm

Table 6

Anise hyssop cultivation experiment in potato ridges
(Puumala, 1989)

Propagation method	Plant density plant/m (a) row/ridge (b)	Plant height cm	weight g/piece	Fresh crop kg/m	Moisture content %	Leaf + flower ratio to dry crop %
	(a)					
Seedling raising	6	69.6	160.8	1.02	79.9	58.5
	10	73.5	106.7	1.06	78.5	61.3
	16	77.3	78.9	1.26	79.2	58.3
p<		xxx	xxx	x	—	ns
	(b)					
Direct sowing	1	73.7	—	1.26	81.7	54.3
	2	76.7	—	2.07	82.6	49.7
	3	76.0	—	1.97	84.0	50.8
p<		ns	—	xx	xxxx	ns

p< 0.1% = xxxx
p< 1 % = xxx
p< 5 % = xx
p<10 % = x
ns = non significant
— = not counted

ing percentage is also very good if the second growth is not used as drug. If in mid-spring (April—May) severe frosts occur without snow-cover, or the crop is cut too late, a considerably higher percentage of the plants perish by frost. In the case of intensive drug production — when the second growth is also harvested — the unreliable overwintering makes it reasonable to raise as many seedlings as sufficient for the annual cultivation of anise hyssop.

Productivity, processing, quality

For the purpose of drug production the plants are harvested at the beginning of flowering and in full blossom, respectively, under the conditions of Finland in the first half of August. In the different experiments more or less the same yields were obtained. In the field stands spaced at 40 × 40 cm the fresh crop ranged from 1.7 to 2 kg/m^2 (Table 5). Owing to the higher plant density (10 plants/m^2) the plots covered with balck polythene gave a yield of 1.6—2.2 kg/m^2. Nearly the same yield was attained with the ridge cultivation of the plants, though because of the distance of the ridges (80 cm) the yield here was naturally smaller (Table 6). The two latter methods proved highly suitable in the case of growing the plants without chemical weed control; both when covering the soil with black polyethylene plastic and when using ridges, we were able to reduce by 80% the manual labour requirement of weed control.

Table 7

Percentage distribution and dry matter content of the main parts of anise hyssop. (Puumala, 1989)

Harvest time	Total plant weight g	% distribution of plant parts				Dry matter content %			
		flower	leaf	stalk	root	flower	leaf	stalk	root
1. 16.7.	23.8	2.2	42.5	35.7	20.6	23.1	22.1	15.6	12.8
2. 06.8.	84.6	11.7	33.7	40.8	13.8	18.8	26.8	20.6	16.0
3. 14.8.	104.0	25.4	27.4	34.4	12.8	19.0	21.6	21.6	16.6
4. 31.8.	113.1	33.2	22.8	30.3	13.7	21.5	21.8	27.0	14.7
5. 12.9.	120.7	34.4	22.5	27.3	15.8	23.4	21.5	27.8	14.2

The anise hyssop plant has a relatively thick stalk and a rich, fibrous root-system. The ratio of the plant parts important for drug production is shown in Table 7. In the course of the vegetation period, the proportion of the root-system is relatively steady, the proportion of leaves and stalk decreases, while the advance of tillering the proportion of the inflorescence increases.

The dry matter content of the biomass harvested in full blossom ranges between 16 and 28% (Table 7). In the cool and wet August of Finland high quality drug can only be produced by hot-air drying (40 °C). Owing to the relatively high proportion of the thick stalk parts, the harvested raw material dries readily and reliably. The air-dry material is mechanically crumbled, then the stalk parts are separated from the leaves and inflorescence by winnowing. The ratio of the leaf and flower parts of commercial value to the total dry crop ranged between 60 and 70%. The volatile oil content of the dry drug thus produced is 0.1—0.3%, and according to the results of the laboratory analyses, the main components of the volatile oil are: methylkavikol, 74.6%; limonen, 8.5%; β-karyophyllen, 5.5% and germarene-D, 3.3% (Table 8).

According to the results of heavy metal analyses performed in the Central Laboratory of the Agricultural Research Centre the lead and cadmium contents of the anise hyssop drug produced at 8 experiment stations of the country were very low. We found the lead content to be 0.51 ppm, and the cadmium content to be 0.011 ppm (Table 8), (Galambosi and Kumpulainen, 1990).

Summary

Between 1986 and 1990 detailed cultivation experiments were carried out with the perennial anise hyssop (*Agastache foeniculum* (Pursch) Kuntze) plant of North-American origin. The leaves and inflorescence of the decorative plant developing deep pink flowers have a pleasant odour recalling that of the anise, they are therefore used to season sweets, ice-creams and tea mixtures. The above round parts of the plant contain 0.1—0.3% volatile oil, with methylkavikol as main component.

Under the conditions of South-Finland, the vegetation period of the plant grown in direct sowing or seedling raising system is 95—110 days. The average germinative ability of the seed is 78%. In the case of drug production, overwintering is uncertain. Its production is 100—200 kg fresh and 10—25 kg dry, marketable crumbled drug per 100 m². The plant can be successfully grown with chemical-free cultivation methods, with black polyethylene plastic mulching or on ridges.

Table 8

Composition of the volatile oil anise hyssop
(Nykänen et al. 1989)

Component	%
1. β-Pinene	tr.*
2. Sabinene	tr.
3. 1-Butanol	tr.
4. Myrcene	0.1
5. Limonene	8.5
6. 2-Pentylfuran	0.1
7. 3-Octanone	0.6
8. 1-Octen-3-yl acetate	0.4
9. 1-Octen-3-ol	0.4
10. Menthone isomer 1	0.2
11. Menthone isomer 2	1.1
12. Pinocamphone, isomer 1	tr.
13. Pinocamphone, isomer 2	tr.
14. Linalol	0.6
15. β-Caryophyllene	5.5
16. α-Humulene	0.1
17. Methylchavicol	74.6
18. Germacrene D	0.4
19. Germacrene B	3.3
20. δ-Cadinene	0.4
21. γ-Cadinene	tr.
22. 1, 2, 3, 4, 4a, 7-Hexahydro-1,6-dimethyl-4-isopropylnaphtalene	tr.
23. α-Amorphene	tr.
24. Damascenone	0.1
25. Anethol	tr.
26. Geraniol	0.1
27. Geranylacetone	tr.
28. β-lonone	0.2
29. Caryophyllene oxude	0.2
30. Methyleugenol	0.2
31. Spathulenol, isomer 1	1.1
32. Eugenol	0.1
33. δ-Cadinol, isomer 1	0.5
34. α-Cadinol, isomer 2	0.2
35. Thymol	tr.
36. δ-Cadinol, isomer 2	0.2
37. Carvacrol	tr.
38. Spathulenol, isomer 2	0.1
39. α-Cadinol, isomer 2	0.4

*tr. = traces = <0.1%

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GENETIC AND TECHNOLOGICAL PROGRESS WITH THE MAJOR FIELD CROPS IN THE HUNGARIAN AGRICULTURE

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The genetic progress qualifies first of all the breeding activity, while the technological progress the level of the cultivation practice. It also, explains

— the harmony of breeding and cultivation,
— the adequacy of the conditions of both breeding and cultivation,
— the international level of breeding and cultivation,
— with a complementary analysis to the further duties in the period examined.

In the case of the major field crops — with the exception of potato — the degree of both the genetic and the technological progress has attained or occasionally even exceeded that of the agricultures of economically developed countries. The distance between the genetic and the technological progress does not in itself qualify the technological progress. The causes of the difference can be disclosed by the comparison of the properties and production conditions of the variety/hybrid representing the genetic progress and the circumstances ensured by the cultivation practice.

Keywords: breeding, genetic progress, maize, peas, potato, sugar beet, sunflower, technological progress, technology, winter wheat

Introduction

The quality of the breeding work is determined beyond the large number of Hungarian varieties and their distribution primarily by the properties of the new varieties.

The distinctive properties appearing in the new variety compared to the old one are expressed by the genetic progress. The genetic progress is a surplus quality attained through breeding.

As regards maize, Németh and Kálmán (1987) estimate the genetic progress in Hungary to be 1.2% on the average of the past 15 years. They cite data of Duvick, Russel, Castleberry who show the genetic progress to be 57—75% on the average of the last 32—60 years.

According to Győrffy (1985) in the increase of the average yield of maize from 1.758 to 7.534 t/ha the role of the variety is 26%.

Kapás (1978) studied in Hungary — among others — the efficiency and intensity of the change of potato variety on the basis of data from the period between 1961 and 1973. The indices calculated on the basis of an increase in the yield average show the success of the variety change (11.28%) while the index by

age its intensity (1.14%), and the ratio of the two characterizes the efficiency (10.83%). According to Kapás, in the period examined the intensity of variety change in sugar beet showed a decreasing tendency; while the average age of the wheat and maize varieties at the end of the period concerned was about 6 and 7 years, respectively, in sugar beet production it was 12 years or so.

At Martonvásár the progress of selection was studied in a 6-year series of experiment by comparing the major standard varieties of the past two decades and the new Martonvásári varieties.

The "old" variety, 'Bánkuti 1201' produced at the present agrotechnical level 4.94 t/ha on a six-year average. 'Besostaya 1', the standard variety of the next period produced 6.03 t/ha, 22% more than 'Bánkuti 1201'. The yield of 'Jubilejnaja 50' and 'Martonvásári 4', the two standard varieties of the period following 'Besostaya' 1 was 6.57 and 6.63 t/ha, 33 and 34% more, respectively, than the yield of 'Bánkuti 1201'.

The three Mv varieties — 'Martonvásári 8', 'Martonvásári 9' and 'Martonvásári 10' — produced 7.01, 7.38 and 7.53 t/ha, respectively, under ecological conditions identical with those of the above varieties. These three varieties outyielded 'Bánkuti 1201' by 42, 49 and 53%, respectively. On the basis of these varieties the progress by selection is thus 48%, on an annual average 2.3% (Balla 1987).

On the rate of progress by selection, or genetic progress extremely varying data are found both in Hungarian and in the international literature. It is considered to be 62.3% by Elliot (1962), 60% by Austin (1978) and 50% by Lupton (1982) in the United Kingdom, 33.8% by Schmidt (1975) in Czechoslovakia while in Hungary Bócz (1973) estimates it at 30—40% and Kapás (1978) at 30%.

The genetic progress made by breeders of the USA between 1958 and 1980 was 0.74%/year (Schmidt, 1985).

In the Hungarian relevant literature the concept of technological progress is almost unknown. According to Reinken (1982) the technological progress is a result attained in increasing the fertility of plants and animals using new methods, machines, implements and a new mode of cultivation. Related to West-Germany its rate is 75 kg/year for wheat, 130 kg/year for maize, 410 kg/year for potato and 480 kg/year for sugar beet (1950—1980).

Klein and Kehrberg (1981) give the following definition for the technological progress: higher output from the same resources or the same output produced from less resources. This abstract definition expresses the nature, while the former one the means of the technological progress.

In crop production the technological progress can be expressed by the difference in yield average between two periods related to one year. In the Hungarian literature this is called the growth rate of yield average (Balla, Győrffy, Németh, Hajdú etc.). The term technological progress is, however, a more exact and more correct definition as it also refers to the source of the growth of yield average.

Materials and methods

For measuring the genetic progress the data of the Hungarian variety trial stations were processed. Productivity is expressed by the Maximum Yield Average Realizable in Practice. This value is the one that the farmer can set as a rational target of production. Considering the diverse natural conditions of Hungary, from the data of variety trials the 6 highest values of the given variety were taken into account at each growing site, generally on the average of 3 years. With this method the genetic progress was calculated for the period between 1950 and 1985: on the basis of the initial data of Bánkuti 1201 for wheat, of Mezőhegyesi F lófogú for maize, of Béta K-91 for sugar beet, of Lovászpatonai for sunflower, of Express for peas, of Ella for potato. To these were the data of varieties/hybrids in periods of technological change compared (Hajdú, 1987, 1990).

The genetic progress can, however, be related to properties other than productivity as well (gluten content, resistance etc.). In this respect the data of MMI are analysed.

The technological progress is analysed on the basis of the national yield average data of the Central Statistical Office.

Results and conclusions

Genetic progress

For the major field crops the genetic progress is shown in Table 1. Its value is 145.4 (10.5%) for winter wheat, 226.2 (11.1%) for maize, 48.1 (5.9%) for sunflower, 57.3 (7.2%) for peas, 810.6 (6.0%) for sugar beet, 624 (7.7%) for potato.

In consequence of the production policies of the past decades, which encouraged the quantitative development, the breeders attained excellent results. The breeding work satisfied the demands of the Hungarian large-scale production. Partly this and partly the low initial level explain the great genetic progress which even in international relations is outstanding, especially in wheat and maize breeding (10—11% genetic progress compared to 1—2% in foreign countries). The latter also explains the fact that in spite of the excellent results Hungary is still not a leading country as regards the yield averages of wheat and maize.

The rate of genetic progress varies with the crop and from period to period, too. Wheat breeding practically met the domestic demand in the second half of the 1970s, from then on the development has been dynamical. In the case of maize, on the other hand, the success of the work of breeding has for several decades gradually increased. With sugar beet, after the initial success of domestic varieties foreign varieties took over the leading role. The situation is similar with the potato. Sunflower and peas are plants of a type of assimilation different from the former plant. Yet, the result is outstanding again.

As a regards the quality parameters, the progress is not so unequivocal, particularly with wheat and maize. In the case of wheat, with the varieties bred since 1971 taken into consideration, the increase in thousand-grain-weight (0.117) is favourable, but the hl-weight (−0.145), the hardness (−0.888%) and the wet gluten (−0.208%) have decreased. Quality deterioration has taken place with maize too, as both the crude protein and the crude fat content have become lower (by 0.048 and 0.027%, respectively). In the case of sugar beet the digestion shows a slight improvement (0.005%). The thousand-seed-weight of sunflower has dimin-

Table 1

*Genetic progress**

Species (variety)	Period	Yield average t/ha	Annual growth kg	Annual growth %
Winter wheat				
Bánkuti 1201	1950—52	2.29	—	—
Besostaya 1	1960—62	3.85	141.8	
Jubilejnaja	1971—73	4.78	77.5	
Martonvásári 8	1978—80	5.87	136.2	
Martonvásári 14	1983—85	7.38	251.7	
1950/52—1983/85			145.4	10.5
Maize				
Mezőhegyesi F	1948—50	2.94	—	—
Mv—1	1960—62	5.31	197.5	
MvTC 596	1971—73	8.00	244.5	
Szegedi SC 444	1979—82	11.18	353.3	
1948/50—1979/82			226.2	11.1
Sunflower				
Lovászpatonai	1948—51	1.52	—	—
Kisvárdai	1960—62	2.64	101.8	
Iregi csikos	1971—73	3.43	71.8	
Iregi H	1983—85	3.30	—	
1948/51—1983/85			48.1	5.9
Peas				
Expressz	1955—56	1.46		
Petit provensal	1960—62	1.57	22.0	
IP 3	1971—73	2.68	925.0	
Ujmajori arany	1978—80	2.66	—	
IP 3	1983—85	3.18	104.0	
1955/56—1983/85			57.3	7.2
Sugar beet				
Béta K 91	1950—52	25.36		
Béta-poly 4	1960—62	34.45	909.0	
Béta-poly M102	1971—73	45.13	970.9	
Kavepura	1983—85	53.73	860.0	
1950/52—1983/85			810.6	6.0
Potato				
Ella	1950—51	12.7		
Aranyalma	1960—62	24.5	475	
Keszthelyi s.	1971—73	31.46	633	
KZ 1004	1983—85	34.55	25	
1950/51—1983/85			624	7.7

* Calculations by M. Hajdú

ished (−0.4112), the oil content, on the other hand, has considerably improved (0.503%).

Since 1971 breeding institutes of Hungary have produced 35 wheat varieties (3 of them are durum), which include varieties and experimentals with more favourable qualitative parameters.

Table 2

*Tendencies in changes in the biological basis**

Designation	Number of certified varieties			Varieties per 100 000 ha	Variety older than 10 years	Av. age of certified variety	% growth of yield average by 1954 1950 = 100 %
	1950	1978	1985				
Winter wheat	6	17	25	1.9	8.8	5.3	232.6
Maize	13	30	26	2.3	—	3.72	300.5
Sunflower	4	5	18	5.6	16.6	5.3	174.0
Sugar beet	7	11	11	10.0	27.2	6.0	212.0
Lucerne	7	11	13	3.9	53.8	14.0	134.5
r				0.57	0.86	0.78	

* Calculation by M. Hajdú

The dynamical genetic progress leads to a more rapid change of variety, and the average age of the varieties cultivated gradually decreases. Between the average age of the varieties and the increase in yield average there is a close correlation (−0.78); and the correlation between the proportion of varieties older than ten years and the increase in yield average is still closer than that (−0.86).

A structure of varieties with higher age results in lower yield average. The age of the varieties is thus a factor that determines the trend of the yield average (Table 2).

This fact does not contradict to the extreme genetic value of those varieties which are able to satisfy the demand of production over a long period (e.g. the winter wheat variety Mv-4 of Martonvásár, etc.).

The efficiency and nature of the genetic progress are thus motivated and ensured by the existing socio-economic background too. To improve the conditions of the breeding work is, therefore, a basic interest of the farm.

Technological progress

The technological progress in the different regions is shown in Table 3.

On a world average the yields of wheat and maize increased the most dynamically, while this value is lowest for beans, peas, oats and sugar beet.

The rate of increase in the yield averages of wheat and maize compared to the European average was also the highest (some one and a half-times the world average), and dynamical progress was made in the case of early beans too. Relatively less favourable is the rate of increase for peas and rice.

Table 3

*Growth rates of yield averages in major field crops**

Designation	World average			European average			Hungary		
	1948/52	1984	1984 as % year of 1948/52	1948/52	1984	1984 as % year of 1948/52	1948/52	1984	1984 as % year of 1948/52
	t/ha			t/ha			t/ha		
Wheat	0.99	2.25	6.6	1.47	4.71	9.4	1.38	5.21	++11.0
Rye	0.96	1.79	5.4	1.46	2.86	5.6	1.24	1.38	−3.5
Barley	1.13	2.18	5.6	1.69	4.25	7.2	1.44	3.47	+6.9
Oats	1.14	1.69	4.3	1.60	3.18	5.6	1.21	3.25	7.3
Maize	1.59	3.40	6.2	1.24	5.27	12.5	1.77	5.77	+9.4
Rice	1.63	3.18	5.6	4.30	5.18	3.7	2.51	2.30	−2.6
Sugar beet	21.8	33.11	4.3	25.0	42.31	4.4	15.4	39.25	++7.2
Potato	10.8	15.37	4.3	13.7	20.28	4.2	6.8	16.21	++6.5
Early beans	0.44	0.59	3.9	0.23	0.63	7.4	0.6	1.36	+6.3
Early peans	0.86	1.22	4.1	1.28	1.63	3.7	0.94	2.17	++6.5

− = Growth below world average for rye and rice
\+ = Growth above world average but below European average for barley, maize and beans
++ = Growth above world- and European average for wheat, sugar beet, potato and dry peas

* Calculations by M. Hajdú

In the case of rye and rice the increase in Hungary is below world average, while with barley, maize, beans it is above world average, though has not reached the average of Europe. There is an increase above both world and European average in the case of wheat, sugar beet, potato and dry peas.

The technological progress in terms of kg/ha/year for four crops of Hungary compared to 3 European countries is shown in Table 4.

As regards production level Hungary was in 1950 behind France, while more or less kept abreast with Italy and Spain. In France the wheat and sugar beet programme showed outstanding results from 1960, while the maize programme in the second half of the 1960s. In Italy mainly the development of maize production has been spectacular from as early as the 1950s. In Spain from 1971 the maize programme, from 1976 the development of sugar beet production has been dynamical. There are thus differences in both the rate and the time of the technological progress between Hungary and other countries. The main growth phase of agriculture in Hungary — unlike the economically developed countries — coincided with the period of the first oil crisis.

Comparison of the genetic and technological progress

In a comparison between genetic progress and technological progress the value of the latter is always below that of the former. In the period concerned the percentage ratio of the technological to the genetic progress was: 37% for sunflower, 36% for maize, 42% for potato, 73% for sugar beet, 70% for winter wheat.

Table 4

*Technological progress kg/ha/year**

	Sugar beet				Maize			
	H	F	S	I	H	F	I	S
1951—55								
1956—60	500	800	480	240	50	120	116	64
1961—65	720	1100	0,0	160	64	34	58	24
1966—70	1520	1080	320	820	124	350	158	92
1971—75	120	100	720	720	188	10	292	198
1976—80	200	220	1020	1200	136	50	198	114
1951—1980	936				118.9			
Technological progress as % of genetic progress	73				36			

	Sunflower			Wheat			
	H	I	S	H	F	I	S
1951—55							
1956—60	10	22	8	50	10	0.0	12
1961—65	—	46	54	68	142	48	8
1966—70	32	32	34	120	100	56	36
1971—75	24	0.0	0.0	188	172	56	34
1976—80	66	0.0	24	136	64	0.0	66
1951—1980	24.3			102.3			
Technological progress as % of genetic progress	37			70.3			

H = Hungary, F = France, S = Spain, I = Italy

* Calculations by M. Hajdú

The reasons for the difference between genetic and technological progress are:
— the variety structure of the cultivation practice is not identical with the variety taken into consideration when measuring the genetic progress, since cultivation only can follow after the work of breeding. The percentage data can thus indicate the pace of the variety change too;
— the degree of propagation, the quality of the seed tuber is not the same in the variety trial as in the cultivation practice;
— there are differences in the other technological elements too (e.g. fertilization, sowing, tending, harvesting, etc.).

On the basis of the percentage data presented the data may mean that
— the technology satisfies the demand of the variety/hybrid, so the exploitation of the production potential is favourable, or
— the technology is not in harmony with the demand of the variety/hybrid;
— in consequence of a slow change of variety the rate and result of a technological modernization render the exploitation of the production potential favourable;
— owing to the quick change of variety the technology has not yet met the demand of the variety/hybrid;

— owing to the slow change of variety, practice does not make use of the possibilities inherent in the production potential.

In the case of wheat and sugar beet the rate of technological modernization has resulted in a favourable exploitation of the production potential, while in sunflower, maize and potato production the technology has not yet met the demands of variety/hybrid, owing to a relatively rapid change of variety.

In general, the genetic progress represents a pulling power for the technological progress. However, the history of crop production proves that the technological progress may as well force out the genetic progress, as shown by the example of wheat in the United Kingdom, where from the middle of the 1960s to the end of the 1970s the yield average of wheat increased but slightly (4—5 t/ha). The more dynamical increase beginning with the 1980s is connected with the variety change and a technology that satisfies the demand of variety. The Hungarian wheat production has been in a similar situation since the 1980s.

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Plant Genetics and Breeding

GYNOGENIC HAPLOIDS PRODUCED IN OVULE CULTURES OF MALE STERILE, FERTILE, MONO- AND MULTIGERM SUGAR BEET (*BETA VULGARIS* L.) LINES

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In *in vitro* cultures of ovules removed before anthesis from closed flowers of male sterile and fertile breeding materials haploid plants were produced by a single-step procedure. In the course of the haploid induction the best result was attained with a modified MS culture medium supplemented with 5 μM/l BAP and 1 μM/l NAA, or with 5 μM/l BAP and 5 μM/l NAA. The maximum plant regeneration was 18%. The average frequency of gynogenesis was 0.5—1.5%, its maximum frequency 10%, which showed a great variability not only between the lines but also between the plants within a line. Genotypic dependence was particularly expressed in the fertile lines. The culture medium inducing gynogenesis has no unambiguous effect on the subsequent propgation rate. In the course of the first three subcultures the propagation rate fell from 9x to 4.5x.

Keywords: gynogenesis, micropropagation, ovule culture, sugar beet

Introduction

Production of homozygous lines by inbreeding in the biennial allogamous sugar beet takes a long time. Haplo-diploidization makes it possible to produce homozygous lines from a given genotype in a short time.

Various methods have been tested so far to produce haploid plants in sugar beet (Jensen, 1986). *In vitro* gynogenesis seems to be the most promising of them, which can be induced in *in vitro* cultures of unpollinated ovaries and ovules. Hosemans and Bossoutrot (1983) were the first to observe callus and embryo development in 2.1% of ovules, and 0.23% of the regenerated plants proved to be haploid. Later D'Halluin and Keimer (1986) even attained 26.6% frequency with certain genotypes. Doctrinal et al. (1989) using a two-step procedure obtained 6—10% viable haploid plants in male sterile breeding materials. The results attained until the end of the eighties were summarized and evaluated in an earlier paper (Potyondi and Heszky, 1991).

The frequency of gynogenesis is lower in fertile plants. In the case of fertile plants Bossoutrot and Hosemans (1985) tried to ensure suitable isolates by castra-

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tion using self-incompatible genotypes. Since, however, in the sugar beet flowers the embryo sac develops fully some 6 days before the dehiscence of the anthers, D'Halluin and Keimer (1986), Van Geyt et al. (1987), Lux et al. (1990) carried out successful experiments with cultures of ovules removed from fertile, still closed flowers.

On the basis of the above, using the single-step method in our experiments, we wished to study and prove — besides the applicability of the single-step method — the differences in the induction of gynogenesis partly between the different lines, partly between the plants within the lines.

Materials and methods

Lines examined

The experiments were performed in 1988 and 1989 with one male sterile monogerm line (cms 85—588), 4 fertile monogerm lines (E-101, M-1, H-1, H-54) and the L-122 multigerm line. We used flower buds from more than one plants per line.

The male sterile plants were grown in isolation boxes, the others under field conditions. The ovules were isolated from closed flowers which had not yet reached the stage of anther dehiscence, from the end of May to the end of July. The flower buds were sterilized by shaking in 0.04% $HgCl_2$ solution for 30 minutes, then washed 3-times with water.

Culture media used

For the induction of gynogenesis a solid MS basic culture medium modified by Nakashima et al. (1986), supplemented by 30 g sucrose (mMS) was used; its pH was 8. The culture medium was completed with benzyl aminopurine (BAP), and naphtyl acetic acid (NAA) at concentrations of 1—5 μM/l.

For subculturing and *in vitro* micropropagation B-5 (Gamborg and Eveleigh, 1967) basic culture medium containing 0.5 mg/l BAP and 0.01 mg/l 2,4 D (2,4 dichloro-phenoxy acetic acid) was used.

The ovules were incubated in dark at 25 ± 1 °C. Following the appearance of shoot primordia the cultures were kept under 24-hour illumination at 22 ± 1 °C. The cultures were micropropagated and maintained under similar conditions.

Ploidy level determination

For the transplanted and *in vitro* micropropagated clones the ploidy level was examined using 2 techniques.

The chromosome number in cells of the shoot apex was determined with the carmine acetic acid method elaborated at our institute and applied in the work of breeding (Varga, 1961).

The number of chloroplasts in the guard cells of stomata was determined by the method of Deuter (1970) and Hosemans—Bossoutrot (1983) applying staining with Lugol solution.

Results and discussion

Induction of gynogenesis in sterile and fertile lines

From sterile (cms 85—588) and fertile (H-54, E-101) monogerm lines a total of 955 unpollinated ovules were isolated to four kinds of MS culture media (Table 1).

On the average of the experiment from 1.2% of the isolates shoot, from 0.9% root developed, and 0.8% became callused. On the 7th to 19th day following the

Table 1

Haploids produced by gynogenesis from sterile and fertile monogerm sugar beet lines

Breeding line	Culture medium	Isolated ovule	Time of appearance of first	Induced			Regenerated	Regenerated haploid	
				shoot	root	callus	plant	plant	
		n	organs day	n	n	n	n	n	%
cms 85—588	I1	100	35 (S)	1	—	—	1	1	1
	I2	100	19 (R)	—	—	1	2	—	—
	I3	100	21 (S)	4	2	—	3	3	3
	I4	100	51 (S)	1	—	1	1	—	—
H—54	I1	67	93 (S)	1	1	—	1	—	—
	I2	68	13 (C)	1	—	1	1	1	1.5
	I3	61	7 (R)	1	1	2	3	—	—
	I4	63	140 (C)	—	—	1	1	—	—
E—101	I1	59	—	—	—	—	—	—	—
	I2	59	90 (C)	—	—	1	—	—	—
	I3	67	30 (S)	—	—	1	—	—	—
	I4	57	—	—	—	—	—	—	—

Abbreviations: S = shoot, R = root, C = callus
Culture medium: I1 = mMS I3 = mMS + 5 μM/l BAP + 1 μM/l NES
I2 = mMS + 5 μM/l BAP I4 = mMS + 1 μM/l BAP + 5 μM/l NES

isolation the roots, then on the 21st to 93rd day the shoots, finally after 3—4 months, the callus appeared in the cultures.

A total of 13 plants was regenerated of which 5 (38%) proved haploid. After all, the frequency of gynogenesis was 0.5% of the number of ovules isolated. There were, of course differences between the culture media employed and the breeding lines used, but they cannot be regarded as significant (Table 1).

Our results agree with those published by D'Halluin and Keimer (1986) and Lux et al. (1990), namely, that from ovules of flowers isolated before anthesis haploids can be regenerated in the case of both male sterile and fertile plants.

The fact that haploids could even be produced on hormone-free culture medium proves the deficiency of knowledge related with the hormonal regulation of the induction of *in vitro* gynogenesis, which is supported by the results of Nakashima et al. (1986) too.

Further, the results of Table 1 show that from male sterile lines haploids can be regenerated relatively easier and with higher frequency, than from fertile ones. We, therefore, describe below the results connected with the latter.

Induction of gynogenesis in fertile mono- and multigerm lines and plants

From 3 plants of each of two monogerm lines (M-1 and H-L) and from a multigerm line a total of 894 unpollinated ovules were isolated onto five kinds of mMS culture media (Table 2).

On the average of the experiment shoot developed from 2.4% of the isolates. Plants could be regenerated from 36% of the shoots, and they were 100% haploids.

There were considerable differences in the frequency of gynogenesis not only between the mono- and multigerm lines but also between the plants within the

monogerm lines. Among the plants of the relatively readily regenerating M1 line the frequency of induction ranged from 0 to 10%, which agrees with the result obtained by Doctrinal et al. (1989) on male sterile lines. These results prove that in the case of fertile lines the extent of the induction of gynogenesis shows a high genotypic dependence. In fact, the breeder has to find those cuttings within the lines from which haploids can be produced in a sufficient number with relative easiness (see Table 2, M-1/3 plant).

Table 2

Effect of culture medium and genotype on the in vitro gynogenesis of various fertile mono- and multigerm sugar beet lines

Breeding line/plant	Culture medium	Isolated ovule n	Time of appearance of first shoot day	Induced shoot n	Regenerated plant n	Regenerated haploid plant n	Regenerated haploid plant %
M—1/1	I1	52	106	2	1	1	1.9
	I3	91	42	5	1	1	1.1
	I5	10	—	—	—	—	—
	I6	10	56	1	—	—	—
M—1/2	I1	54	—	—	—	—	—
	I3	92	—	—	—	—	—
	I5	20	—	—	—	—	—
	I6	20	—	—	—	—	—
M—1/3	I1	33	—	—	—	—	—
	I3	30	33	3	2	2	6.6
	I5	20	31	3	—	—	—
	I6	20	28	5	2	2	10
H—1/4	I1	16	—	—	—	—	—
	I3	59	41	1	1	1	1.7
	I5	10	—	—	—	—	—
	I6	10	—	—	—	—	—
H—1/2	I1	20	—	—	—	—	—
	I3	65	—	—	—	—	—
	I5	20	46	2	1	1	5
	I6	21	—	—	—	—	—
H—1/3	I2	21	—	—	—	—	—
	I3	80	—	—	—	—	—
L—122	I1	62	—	—	—	—	—
	I2	41	—	—	—	—	—
	I3	19	—	—	—	—	—

Culture medium: I1 = mMS, I2 = mMS + 5 μM/l BAP, I3 = mMS + 5 μM/l BAP + 1 μM/l NES, I5 = mMS + 5 μM/l BAP + 2.5 μM/l NES, I6 = mMS + 5 μM/l BAP + 5 μM/l NES

Of the culture media those stood the best which contained NAA besides BAP. However, the extent of their effect greatly varied with the line and plant. Further investigation are required to elaborate universal culture media and methods.

As opposed to the results of D'Halluin and Keimer (1986), and Doctrinal et al. (1989) the auxin level of 3 μM or higher did not always prove unfavourable for the gynogenesis (Table 2).

Table 3

In vitro micropropagation of gynogenetic haploids of various origin

Genotype/ haploid clone		Original culture medium	First transoculation			Second transoculation			Third transoculation		
			time	shoot number	propaga-tion rate	time	shoot number	progpaga-tion rate	time	shoot number	progpaga-tion rate
		*	day	n	x	day	n	x	day	n	x
cms	/1	I1	30	16	16	28	82	5.1	68	468	5.7
85—588	/2	I3	28	15	15	31	96	6.4	67	779	8.1
	/3	I3	51	5	5	77	20	4	88	60	3
	/4	I3	52	1	1	76	5	5	81	40	8
H—54	/1	I2	55	4	4	78	22	5.5	70	30	1.4
M 1/1	/1	I1	27	15	15	36	39	2.6	43	242	6.2
	/2	I3	71	10	10	70	29	2.9	48	126	4.3
M 1/3	/1	I3	70	15	15	72	17	1.2	48	33	1.9
	/2	I3	72	2	2	71	16	8	48	60	3.7
	/3	I6	32	14	14	44	72	5.1	63	390	5.4
	/4	I6	71	13	13	70	57	4.4	43	184	3.2
H 1/1	/1	I3	39	6	6	36	14	2.3	35	57	4.1
H 1/2	/1	I5	70	1	1	36	14	14	35	46	3.2

* For composition see Tables 1 and 2

In vitro propagation and maintenance of haploids

For the maintenance and propagation of haploid plants produced by gynogenesis the *in vitro* vegetative micropropagation method generally used for sugar beet was employedd. With this technique the new shoots develop from the axillary buds of the leaves.

Table 3 shows the cloning data of 13 haploid plants of different origin in the first three propagation cycles. It can be seen that the hormone composition of the induction medium has no definite influence on the rate of multiplication. The greater is the influence concerning the origin of the haploid clone. In the first propagation cycle it was 9×, in the second 5.1×, in the third 4.5×. For the different haploid clones these values ranged from 1.0 to 16.0, from 1.2 to 14.0 and from 1.4 to 8.1, respectively, in the successive subcultures.

The haploid level did not change in the course of propagation. The method is thus suitable for propagation gynogenetic haploids unrestrictedly and maintaining them without time limitation.

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Plant Protection

PROTECTION OF STORED *ZEA MAYS* L. AGAINST *SITOPHILUS ZEAMAIS* L. WITH NON-TOXIC NATURAL PRODUCTS: POTENTIALS OF *XYLOPIA AETHIOPICA* AND *PIPER GUINEENSE*

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The fruits of two aromatic plant species, *Xylopia aethiopica* and *Piper guineense* were evaluated for their insecticidal activity on *Sitophilus zeamais*. The insect was exposed to various levels of the dust or ethanol extract of the dry fruits of the test plants in Petri dishes, each containing 25 g of *Zea mays*. Significant insect mortality was observed in treated grains from 48 to 96 hours after treatment. Activity of the extracts was more rapid than that of the dusts.

Keywords: insecticides, maize, *Piper guineense*, plant protection, *Sitophilus zeamais*, *Xylopia aethiopica*, *Zea mays* L.

Introduction

Cereal grains such as maize and rice are usually attacked in storage by a number of insects. The females of these insect pests lay their eggs in or on the grains and the larvae and some adults cause serious losses by feeding on the grains. In the case of serious infestation, up to 90% of the grain may be destroyed within six months (Rouanet, 1987). Popular grain protection chemicals are potentially dangerous and have variously been implicated as being general environmental pollutants or posing public health hazards (Oudejans, 1982; Lindbald, 1978). The potential toxicity of many conventional grain protection chemicals becomes a matter of major concern in the developing countries where a majority of pesticide users are illiterate or ignorant of the hazards of these chemicals.

Both ecological and economic factors dictate that small-scale farmers in developing countries should be very sparing in their use of pesticides and other chemicals (Strong, 1989). Gill (1971) had earlier suggested the use of non-toxic protectants as an ideal solution to the problem of toxicity and environmental pollution posed by conventional pesticides.

Many plant species are known to possess inherent biochemical substances for self-protection in the competition for survival in the ecosystem. Some of these substances are utilized in allelopathy by the donor plants or act as repellents or toxicants to pests. Numerous floristic studies also indicate that a wide range of

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useful substances, mainly pesticides, pharmaceuticals and flavours are synthesized by a wide spectrum of plant species (Crocomo et al., 1981).

In Nigeria and many other developing countries, the crude use of many plant species, notably, *Xylopia aethiopica, Piper guineense, Ocimum viridis* and *Capsicum* sp. as both pesticides and local medicines has been variously reported (Irvine, 1961; Keay et al., 1964; Ayensu, 1978; Dupriez and DeLeener, 1989). Olaifa and Erhun (1988) reported the use of oils and powder of *P. guineense* for *C. maculatus* control. In this research, therefore, we tried scientifically to appraise the potentials of two local aromatic plants, *Xylopia aethiopica* and *Piper guineense* in the protection of stored maize against the maize weevil, *Sitophilus zeamais*.

Materials and methods

Preparation of the Candidate Insecticides

Dust:

Ripe fruits of *P. guineense* and *X. aethiopica* were sun-dried, ground separately in an electric blender, and sieved with a 1 mm mesh screen to obtain the dust.

Extract:

90 g of *P. guineense* dust was exhaustively extracted in 375 ml of 95% ethanol (EtOH) by Soxhlet-apparatus to give a stock concentration of 0.24 g/ml (calculated 375/90 g/ml = 0.24 g/ml). Similarly, 88 g of *X. aethiopica* was extracted in 475 ml EtOH to give 0.185 g/ml of stock solution.

Treatment procedure

Dust of *P. guineense* or *X. aethiopica* was added to Petri dishes containing 25 g of maize at four levels, 0.0 g, 0.1 g, 0.2 g and 0.3 g. The Petri dishes were thoroughly shaken for effective contact of the grains with the dust. Ten newly-emerged (12—48 h old) *S. zeamais* were introduced in each Petri dish and covered. Each treatment was replicated four times. In a second trial, 1 ml each of *P. guineense* or *X. aethiopica* extract was applied to 25 g of maize in Petri dishes at three levels, 0% (untreated control), 50% and 100% (stock), and exposed to a gentle air blast from an electric fan to allow a complete evaporation of the alcohol. Fifteen minutes later, ten *S. zeamais* (12—48 h old) were introduced in each Petri dish and covered. Again there were four replicates of each treatment level.

Dead insects were counted at 12-hour intervals. Percent (%) mortality and corrected % mortality were calculated from the formulae:

$$\% \text{ mortality} = \frac{\text{No. of dead insects}}{\text{total No. of treated insects}} \times 100$$

$$P_T = \frac{P_0 - P_c}{100 - P_c} \times 100$$

where P_T = corrected % mortality
P_0 = observed % mortality
P_c = control % mortality (Busvine, 1971).

Data collected were subjected to analysis of variance tests and treatment means separated using the Least Significant Difference (LSD) test (Steel and Torrie, 1980).

Table 1

Effect of different concentrations of Piper guineense and Xylopia aethiopica dust on mortality of Sitophilus zeamais in 25g of maize grain

Treatment	Conc. (g)	% Mortality		
		48h	72h	96h
P. guineense	0	0	5	7.5
	0.1	22.5 (22.5)*	52.5 (50.0)	82.5 (81.0)
	0.2	40.0 (40.0)	75.0 (73.7)	97.5 (97.3)
	0.3	35.0 (35.0)	90.0 (89.5)	100.0 (100.0)
X. aethiopica	0	0	7.5	12.5
	0.1	15.0 (15.0)	50.0 (45.9)	75.0 (71.4)
	0.2	25.0 (25.0)	70.0 (67.6)	100.0 (100.0)
	0.3	52.5 (52.5)	97.5 (97.3)	97.5 (97.0)
	$LSD_{5\%}$	14.7	22.8	12.1

* Figures in parentheses show corrected % mortality.

Table 2

Mortality of Sitophilus zeamais exposed to Piper guineense and Xylopia aethiopica extracts in 25g of maize grain

Treatment	Conc. (%)	% Mortality	
		48h	60h
P. guineense	0	5	15
	50	80 (78.9)*	95 (94.0)
	100	100 (100.0)	100 (100.0)
X. aethiopica	0	5	7.5
	50	72.5 (71.0)	77.5 (75.7)
	100	80.0 (78.9)	97.5 (97.3)
	$LSD_{5\%}$	21.2	15.8

* Figures in parentheses show corrected % mortality.

Results

Data presented in Table 1 show that at 48 hours after treatment (h.a.t) highest insect mortality of 52.5% occurred in grains treated with 0.3 g of *X. aethiopica* dust, while 0.3 g *P. guineense* produced only 35% mortality, a value less than the 40% mortality recorded with 0.2 g.

In the trial with the plant extracts (Table 2) a higher mortality rate was obtained with *P. guineense* (100% mortality) than with *X. aethiopica* (80% mortality) at 100% concentration, 48 h.a.t. At 50% extract concentration, mortality rates were 80% for *P. guineense* and 72.5% for *X. aethiopica*. The control mortality was only 5% with either *P. guineense* or *X. aethiopica*. 60 h.a.t. mortality of *S. zeamais*

at the three levels 0, 50 and 100%, of *X. aethiopica* was 7.5%, 77.5% and 97.5% respectively; while with *P. guineense*, mortality at the three levels was 15%, 95% and 100% respectively.

Discussion

Laboratory treatment of maize grains with the dust or EtOH extracts of *P. guineense* or *X. aethiopica* caused significant mortality of adult *S. zeamais* infesting the grains when compared with the untreated controls.

The test toxicants exerted early poisoning symptoms on *S. zeamais*. Within the first few minutes (2—10 min) of contact with the toxicants, the insects showed signs of hyperexcitability. They later appeared dazed, and easily tumbled on movement. These symptoms were more distinct and drawn with the dust where median mortality occurred within 72 h.a.t. In contrast, *S. zeamais* exposed to the extracts died soon after the first signs of hyperexcitability. While 80% of all insects treated with 0.1 g *P. guineense* dust/25 g grain in this study died within 96 h.a.t., Rajapakse (1990) working with *P. nigrum* leaf obtained maximum activity (53.3% mortality) on *Callosobruchus maculatus* at 0.1 g/50 seeds only after 7 days of treatment.

Insect mortality linearly increased with toxicant concentration and duration of treatment. For instance, at the concentration of 0.1 g/25 g of grain, mortality rates caused by *X. aethiopica* were 22.5%, 52.5% and 82.5% at 48 h and 96 h.a.t respectively. At 0.2 g concentration for the same treatment durations, mortality rates were 40%, 75% and 97.5% while at 0.3 g they were 35%, 90% and 100%. The efficacy of *P. guineense* in the protection of stored grains had earlier been mentioned by Ivbijaro (1989) who reported that oil extracts from *P. guineense* adequately protected cowpea seeds in storage against *C. maculatus*.

The fruits of *X. aethiopica* and *P. guineense* are common household spices and medicines in many traditional African communities. To prepare them as pesticidal dust by simple drying and grinding is relatively cheap and practical. They can, therefore, be easily accepted by rural communities as a cheaper and safer alternative to the more expensive and often hazardous conventional grain protectants (Rajapakse, 1990). The candidate toxicants also possess the potentials for the manufacture of ecologically sound pesticides for the pollution-conscious industrialized world.

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Lectures

SALINIZATION AND DESERTIFICATION*

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Among the adverse processes that lead to the deterioration of land and the impoverishment of many nations, desertification and salinization are quite common. The two processes are different, albeit closely interrelated, which means that progressive salinization induces the development of desertification and, vice versa, desertification is commonly associated with increasing salinity.

Consequently, when studying or combating either salinization or desertification the other process, too, should be taken into account because increasing salinization in arid areas always furthers desertification and, on the other hand, in desert areas salinization can, as a rule, hardly be neglected.

Evidently, we are dealing with two processes which are far from being identical, although they are closely interrelated. However, such interrelations are crucial both from theoretical and practical points of view (Szabolcs, 1987).

Extension of desertification and salinization in the world and their interrelations

About one tenth of the surface of the continents is covered by different kinds of salt-affected soils, the majority of which is saline (Szabolcs, 1989).

The distribution of salt-affected soils on the different continents is far from being even, and it is the arid regions that dominate among the salinized areas. Table 1 presents the extension of slightly, moderately, severely, and very severely desertified arid lands of the world (Dregne, 1986).

Dregne (1986) also gave the criteria of estimating the degrees of desertification, which are given in Table 2, from which it can clearly be seen that all the degrees of desertification are associated with a certain degree of salinization, and that a positive correlation exists between the extent of desertification and salinization. This fact also leads to the conclusion that the two processes are in expressed environmental relation.

When we glance at Fig. 1, which shows the desertification of arid lands, and Fig. 2, which demonstrates the global distribution of salt-affected soils, we can see

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Table 1

Desertification of the arid lands of the world

Desertification class	Land area km^2	Percent of arid lands
Slight	24,520.000	52.1
Moderate	13,770.000	29.3
Severe and very severe	8,773.000	18.6
Total	47.063.000	100.0

Table 2

Criteria for estimating degress of desertification

Desertification	Plant cover	Salinization of irrigated land $EC_e \times 10^3$ (mmhos)	Crop yields
Slight	Excellent to good range conditions class	<4	Crop yields reduced by less than 10 percent
Moderate	Fair range conditions class	4—8	Crop yields reduced by 10—50 percent
Severe	Poor range conditions class	8—15	Crop yields reduced by 50—90 percent
Very severe	Land essentially denuded of vegetation	Salt efflorescence on the surface	Crop yields reduced by more than 90 percent

confirmed what has been stated above. However, there are certain differences between the territorial occurrence of desertification and salinization, mainly because salt-affected soils are widely distributed not only in arid and semi-arid regions but also in moderate, subhumid, or even humid climatic belts. Nevertheless, desertification, can mostly be observed in the regions where salt-affected soils also occur. One can say that not all salt-affected soils occur in arid regions but the occurrence of salt-affected soils is frequent or even dominant in all arid areas (Kovda, 1937).

The reason of the joint occurrence of salinization and desertification can also be explained with those geochemical and environmental rules which lead to the development of both processes. It is well known that salinization is the consequence of the accumulation of water soluble salts in the soils, subsoils, surface and underground waters which eventually develops when the climate is dry and the leaching processes are retarded.

Kovda (1980) characterized the salt accumulation processes related to different environmental conditions proving that salt accumulation is in close correlation with aridity as it is demonstrated in Table 3.

Table 3 explains not only the direct correlation of salt accumulation and aridity but also the chemical characteristics of such accumulations in deserts and semideserts where the salinization process mainly occurs and the water soluble salts of sodium and magnesium, partly calcium, dominate.

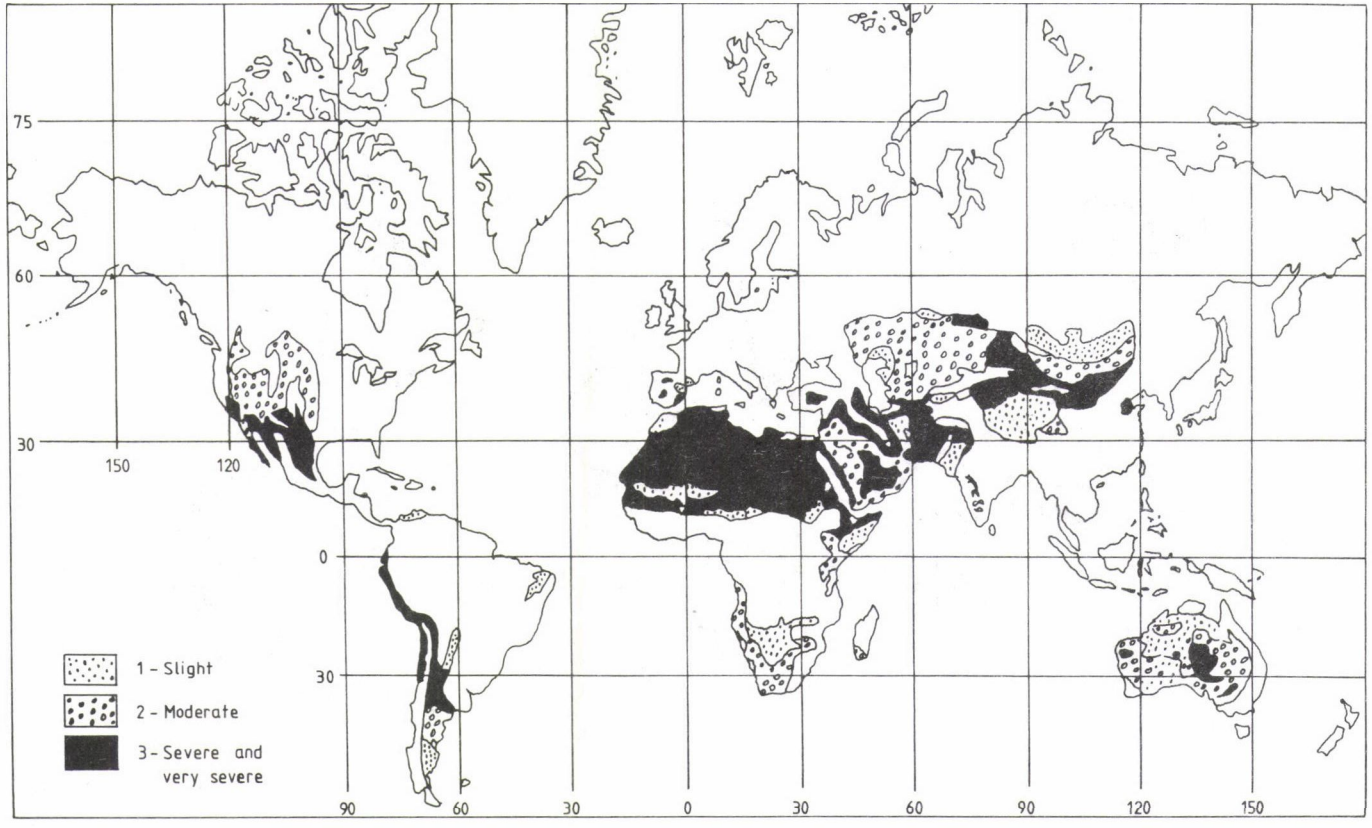

Fig. 1. Desertification of Arid Lands (after H. Dregne)

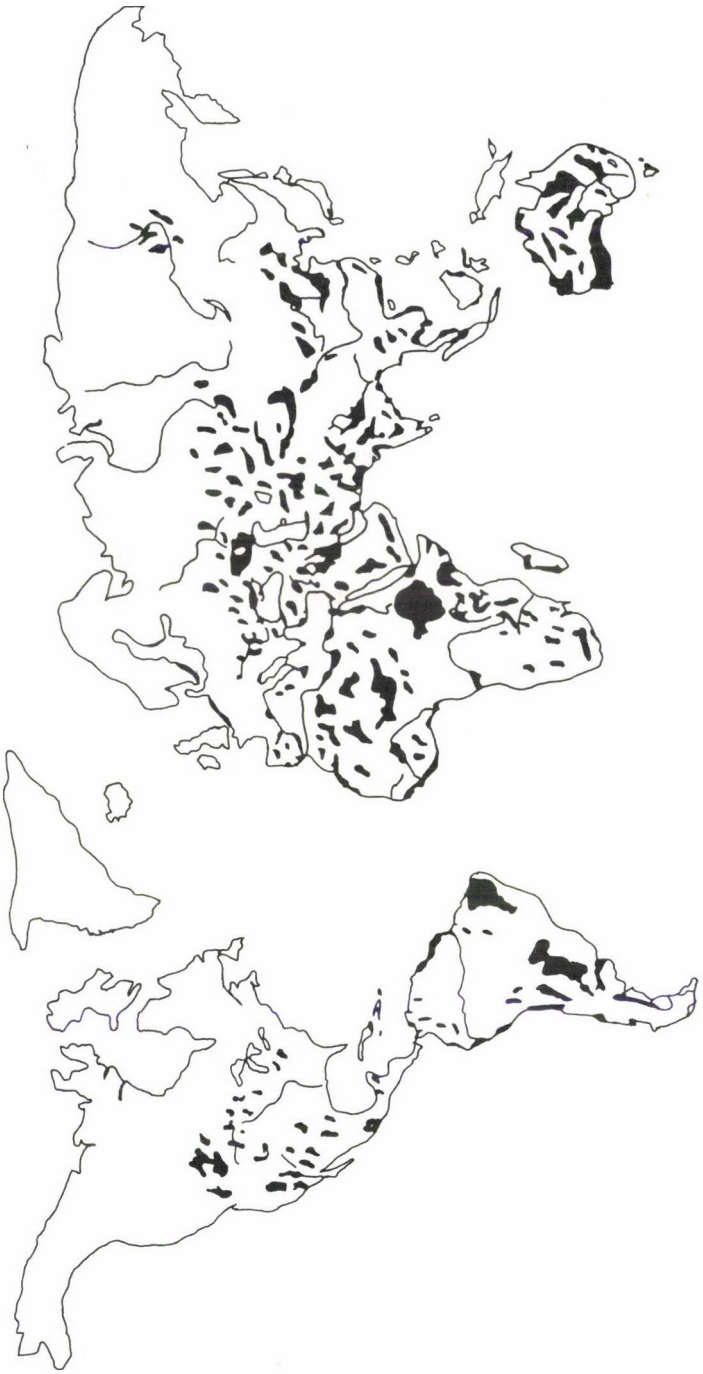

Fig. 2. Global distribution of salt affected soils

Table 3

Characteristics of salt accumulation processes in Eurasia, in relation to natural conditions

Conditions	Residual salinization of sedimentary rocks	Maximum mineralization of waters (g/l)			Maximum % quantity of soluble salts in top horizons of solonchaks	Typical compounds
		River	Ground	Lake		
Desert	Common	20—90	200—350	350—400	25—75	NaCl, KNO_3, $NaNO_3$, $MgCl_2$, $MgSO_4$, $CaSO_4$, $CaCl_2$
Semidesert	Frequent	10—30	100—150	300—350	5—8	NaCl, Na_2SO_4, $CaSO_4$, $MgSO_4$
Steppes	Rare	3—7	50—100	100—250	2—3	Na_2SO_4, NaCl, Na_2CO_3, $NaHCO_3$
Forest steppes	None	0.5—1.0	1—3	10—100	0.5—1.0	$NaHCO_3$, Na_2CO_3, Na_2SO_4, Na_2SiO_3
Forests	None	0.1—0.2	0—1	None	None	R_2O_3, SiO_2

Desertification and salinization are associated not only in noncultivated areas but are also related to human factors as a consequence of improper policy and land use, overloading the environment and neglecting its conservation. It means that besides natural factors socio-economic causes also contribute to the extension of both processes.

For instance, under irrigated farming the salt balance of soils, waters, and underground layers changes and salt accumulation develops, not only on the spot, but often by return flow, infiltratio, etc. in the surrounding areas, far from the irrigated land. As a result of the improper construction and use of irrigation systems, the salinization of the whole ecosystem may ensue in the surrounding areas, furthering desertification.

In many areas salinization and desertification alternately induce each other with disastrous consequences.

The improper methods of irrigation, particularly the lack of proper drainage systems, have resulted in world-wide disaster. According to the data of competent UN associated agencies more than half of all irrigated lands is under the influence of these adverse processes. In spite of the fact that secondary salinization (induced by irrigation) has been well known since ancient times it is expanding in our days at an accelerated rate, particularly in the arid areas of the continents. The total territory of secondarily salinized lands increases by more than 10 million hectares yearly and, in several countries, this results in serious economic problems by devastating the irrigation systems.

A paradox phenomenon occurs frequently when irrigation is introduced as a measure of combating desertification, to mitigate aridity and to increase the yield of arid lands. Namely, when secondary salinization occurs the result is just the opposite in the irrigated fields and particularly in their surroundings: due to salt accumulation desertification intensifies.

All the above factors and considerations lead to the conclusion that the study of, as well as the actions against, either desertification or salinization should be conducted jointly and reciprocally because salinization has at least the following correlations with desertification:

1. salinization promoting desertification
2. salinization developing concurrently with desertification
3. salinization induced by desertification
4. salinization strengthened by desertification.

Table 4

Interrelations between the attributes and consequences of desertification and salinization

Desertification		Salinization
Increase of salt accumulation Decrease of leaching Increase of salt concentration in ground and surface waters as well as in soil layers Secondary increase of water soluble compounds	←	Reduction of water availability Hindering of nutrient uptake Reduction of biota diversity Limitation of plant cover on the soil surface Diminishing of humus content Worsening of thermal and water-physical soil properties
	→	Adverse consequences of irrigation, overgrazing and deforestation

In Table 4 some of the interrelations between desertification and salinization are presented. As it can be seen from Table 4 during salinization and desertification some attributes of the other process appears. For example desertification, as a rule, provokes salt accumulation which is one of the attributes of saline soils. On the other side, e.g., salinization causes a thinning of plant cover on the soil surface which is, in this case, one of the attributes of desertification. Such correlations clearly demonstrate the interrelations between the two processes. During desertification, partly as a result of introducing irrigation, and partly as a result of the degradation of biota, secondary salt accumulation also often occurs.

Present status of desertification with respect to salinization

Rozanov (1990) elaborated a review on the state of world desertification paying attention to most of the arid areas of the world. He interprets desertification in a broad sense including natural and man-made factors, and faces the facts without illusion that the adverse processes are progressing faster than it has been envisaged while the combat against desertification and the improvement of desert lands are more complicated than they seemed to be in the late 70s.

In arid areas the uncontrolled utilization of natural resources, exceeding the limit of ecological stability of natural ecosystems, almost always results in degrada-

tion, which is sometimes irreversible because the appropriate care for the rehabilitation of affected lands is missing (FAO, 1981).

Among the case studies of the Rozanov (1990) report special attention is given to the study of Turkmenia and the Aral Sea Basin (USSR) where the processes of salinization and desertification overlap. In Turkmenia very large areas have been lost to salinization in the wake of the construction of the Karakum canal where around a million hectares became waterlogged and saline. The area of irrigated cropland in eastern Karakum of Turkmenia is nearly 200 thousand ha from which only 140 ha have drainage facilities. Less than 4% of the whole irrigated territory is in a more or less satisfactory state while nearly 90% of all irrigated cropland is heavily damaged by secondary salinization (Pankova et al., 1986).

The situation is particularly gloomy in the basin of the Aral Sea, where all the water of the rivers Syr Darya and Amu Darya is taken, mainly for the irrigation of their valleys, and discharge into the Aral Sea is practically stopped. The present irrigation systems, the industrial, communal, and other consumers use up practically all the fresh water and, at the same time, discharge saline drainage water into the rivers.

Within the last 25 years the water level of the Sea dropped by 13 m, the salinity of the water increased from 9 to 25 g/l and the volume of sea water decreased by more than half (Grigorjev, 1987). In the deltas of the two rivers, creeks and lakes are draining up and the ecosystem changes adversely showing the signs of both salinization and desertification.

Rozanov (1990) also describes in his report other similar adverse phenomena occurring in the Middle Asian Soviet Republics.

In Africa according to Boyadgiev (1984) more than 30% of all agricultural lands suffer from desertification in most cases combined with salinization, altogether half of the whole continent is under the influence of the mentioned adverse processes. Land degradation by salinization and desertification in Sudan, in the Northern and North-Eastern regions of Nigeria, in Ethiopia, Tanzania but even in Zambia, Zimbabwe and Kenya, is dealt with in the Rozanov report.

Very serious problems are reported from the Arab countries of North Africa and South-Western Asia, as well as in Iraq, where, e.g., in Mesopotamia 1 million ha of irrigated land suffer from very severe, 2.2 million ha from severe, 2.3 million ha from moderate and 1.2 million ha from slight salinization making in total 6.7 million ha (Zaletaev, 1989). Furthermore, more than 3 million ha of irrigated land are under the potential danger of salinization.

In Turkey, Pakistan, Syria, the Lebanon and Jordan similar processes have been observed, while in the Arabian Peninsula, which includes Saudi Arabia, North- and South-Yemen, Oman, the United Arab Emirates, Qatar, Bahrein and Kuwait, extreme aridity promotes the development of both salinization and desertification. Egypt, Libya, Algeria, Tunisia and Morocco also suffer from desertification and particularly from the salinization of irrigated lands.

In Iran mainly Khuzistan, the Southern Coastal Plain and the Central Plateau are the scenes of desertification processes, where the areas of range and forest have decreased drastically because most of the country comprises arid and semi-arid

lands where the annual average rainfall is less than 300 mm, sometimes only 50 mm, and the ecosystems are very fragile in the face of degradation processes.

The picture is similarly dark in East and South Africa where desertification affects more than 100 million ha of both rain-fed and irrigated croplands.

In East and South-East Asia the combination of desertification and salinization is also widespread and, particularly in India and China, vast territories of saline desertified land can be identified. In China during the last 15 years the area of desertified land has increased by 3 million ha and its yearly growth exceeds 200,000 ha (Zhao Qiguo and Li Qingkui, 1988). It is expected that if the present process of desertification is not arrested more than 7 million ha will be additionally converted into desert by the end of the century.

In India, particularly in the north-west of the country, but also in the central provinces, desertification and salinization are rapidly increasing, partly induced by improper methods of irrigation and land cultivation.

In North-, Central- and South-America alike, aridity, as a rule, is associated with the processes of salinization and the cultivation of large territories must be abandoned due to these processes.

In Australia, which is the most arid continent, particularly in the central regions, salinity is widely extended and develops concurrently with desertification processes.

Even in Europe, where among all the continents the extent and rate of desertification is the smallest, in the arid regions of the south-east and south-west of the continent salinization and desertification develop jointly and, e.g., in the Trans-Volga region, in Spain and Portugal entail serious environmental and economical problems.

The activity of UNEP and related bodies to combat desertification

Tolba (1984) characterized the essence of desertification as well as those human activities which are responsible for the acceleration of this process.

The United Nations Conference on Desertification was held in 1977 in Nairobi on the initiative of the United Nations General Assembly which took its decision on economic, political and humanitarian grounds, without going deep into the nature of the problem and its magnitude, being deeply impressed by the African drought tragedy. However, the conference was preceded by very extensive studies conducted by a large team of scientists from different parts of the world who assembled and generalized all available facts and relevant information in respect of the problem. These materials, as official United Nations documents, including a series of global maps of desertification, were presented to the Conference for consideration and published later on (United Nations, 1977, 1978; Mabbutt and Floret, 1980; Jain, 1986) as valuable sources of factual information to be used by the world community.

On the basis of carefully collected and analyzed factual materials, the Conference was able to prove the anthropogenic nature of this negative ecological process and large scale of its progressing in arid and semi-arid regions of all continents, its

immediate threat to socio-economic development, particularly in the developing countries of Africa, South and South-West Asia, and Latin America.

Desertification threatens the well-being of 850 million people and embraces an area of some 3.5 billion ha, out of which 3.1 billion ha are represented by pasture lands, 335 million ha by rainfed croplands, and 40 million ha by irrigated agricultural lands. About 21 million ha are annually loosing their productivity due to desertification up to the total economic infeasibility of their use (Rozanov, 1990).

The Plan of Action to Combat Desertification was endorsed in the same year by the United Nations General Assembly, as a large-scale international action programme (United Nations, 1978). The Plan contained concrete recommendations at national, regional and global levels, directed to combating desertification where it is developing and progressing and to reclaiming the biological potential in those areas where desertification has already destroyed the ecosystems.

Consideration of this problem and of the results of international efforts on solving it by the World Commission on Environment and Development under the chairmanship of Norwegian Prime-Minister Gro Harlem Brundtland, which ended its main activity by publishing in 1987 the global assessment of the world and corresponding recommendations for action "Our Common Future" (1987) has led to the conclusion, that desertification is progressing, that the process of desertification affects all the continents of the world, particularly and in the most destructive extent the arid territories of South America, Asia and Africa, where up to 18.5% (870 million ha) of productive lands underwent severe desertification. The Sudano-Sahelian zone of Africa and countries south of it suffer particularly heavily as the process of desertification threatens here the well-being of 80—85% of the total population.

In the same year, 1987, the group of experts of the United Nations Center for Science and Technology for Development had considered the status of desertification and the causes of failure in implementing the Plan of Action to Combat Desertification. The group concluded that after 10 years of trials the Plan needs substantial renovation and reorientation in light of new situations (Land Use Policy, 1987).

In March 1988, at the First Special Session of UNEP's Governing Council, the Second All-System Middle-Term Programme on the Environment for the period of 1990—1995 (ASMTPE-2) was adopted (ASMTPE-1, adopted in 1983 was for the period of 1984—1989) (Kroumkatchev, 1989; Zonn, 1989). In the section of this UN programming document devoted to arid land and desertification, it was noted that, as a result of desertification, 5 to 7 million ha of productive lands are lost annually at present and nearly 21 million ha come into a state of total or almost total economic unfitness. Almost one third of the total land reserve of the world is threatened by desertification, but the most substantially and dramatically in arid and semi-arid regions. Efforts directed to satisfy growing needs in food, together with neglecting the ecological consequences of current methods in agriculture and animal husbandry, enforce the rates of desertification, the main causes of which are overextensive cultivation, overgrazing, deforestation, forest fires and burning of savanna, wind and water erosion, soil salinization. Desertification is closely con-

nected with droughts, which are the results of climatic cycles and are aggravated due to desertification, although it is clearly shown that, by themselves, the climatic droughts do not lead to desertification (Kassas, 1987). Desertification in one region increases the anthropogenic pressure on the natural resources of neighbouring regions, threatening their potentials and increasing the danger of widening the process.

Based on statements and the appropriate international activities in the framework of UNEP, methodology has been elaborated for desertificaton assessment on global level. The main results of this activity are as follows:

1. A world map of desertification was compiled by the responsible UN organization in the scale of 1 : 25 million (FAO, 1981). In the map the different degrees of desertification are delineated. It is supplemented by a series of experimental maps from which, unfortunately, salinity maps are missing.
2. More detailed desertification maps were also elaborated, e.g., for North-Africa and South-West Asia on a scale of 1 : 5 million.
3. A number of technical reports have been periodically published by FAO/UNEP/UNESCO. The methodology was tested in a number of countries on five continents. Pilot studies were also undertaken including the use of satellite remote sensing data in digital format.
4. The activities of UNEP for combating desertification were correlated with and incorporated into several other international and national projects, e.g., with the FAO/UNEP/UNESCO project for the soil degradation maps of Africa, north of the Equator, and the Near-Middle East. It was also correlated with the UNEP/ISRIC project for the global assessment of soil degradation (GLASOD), and with the SOTER project. The latter is concerned with the present status of land resources.
5. The methodology was developed at national level in a number of countries (USA, USSR, etc.).
6. In February 1990 an *ad hoc* consultation meeting took place in Nairobi (Kenya) on the assessment of global desertification. 54 experts participated from different parts of the world. The meeting evaluated the results and delineated future tasks. The report (Rozanov, 1990) was supplemented by country studies from Argentina, North-America, Australia, the USSR, Mali, Mongolia, Afghanistan, Kenya, as well as from Sahelian and Sudanese regions.

The meeting recommended a unified interpretation of terms and approaches as well as information systems, and proposed to prepare a global map of desertification on the scale of 1 : 10 million. It was also recommended to strengthen international collaboration in the study and assessment of global desertification.

The interrelation between desertification and salinization was also discussed at the meeting and the insufficiency of attention paid to salinity when dealing with desertification problems was also mentioned.

The participants of the meeting emphasized the socio-economic aspects and importance of the consequences of both desertification and salinization and that politicians and decision-makers are not sufficiently aware of the grave vistas that will open up when anthropological pressures increase on the natural resources.

Prospects of co-ordinated study and combat of desertification and salinization

From the above it follows that salinization and desertification are so closely interrelated that not only their joint study but also coordinated plans and measures for aborting their further extension seem to be inevitable.

In this respect, as the conclusion of this paper, the following can be recommended:

1. To prepare, parallel with maps of desertification, maps of salinization on the given region or area and to use a combined interpretation of the two maps. Salinity maps are available for most of the continents and regions on scales similar to those of desertification maps. For certain areas, like North-Africa and the Middle East, supplementary mapping of salinity should be carried out in order to facilitate the programmes of combating desertification.
2. Particular attention should be paid to salinity processes when irrigation is planned or practised in regions threatened by desertification processes. As it was described above, in irrigated farming the salt balance of soils, waters and underground layers will change and salt accumulation develops often on the spot but sometimes, by return flow, infiltration, etc. far from the place of irrigation. The higher the rate of salt accumulation is the faster the progress of desertification will be.
3. It is unanimously accepted that monitoring has a first rate importance in spotting and combating desertification. The methods of monitoring salinization are well elaborated and can be applied in combination with the methods of monitoring desertification. Good laboratory methods, limit values and remote sensing patterns are highly applicable.
4. The areas potentially endangered by desertification and salinization, respectively, should be delineated in order to identify the place and rate of the hazard and to take the necessary preventive measures in good time before the adverse processes extend. In this respect the joint study of lands can be highly recommended, because the field, laboratory and other methods for tracing the hazard of secondary salinization are well elaborated, available, and can well be applied jointly with those used for the study of desertification.

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Reviews

MYCOFLORA OF HORSE-BEAN (*VICIA FABA* L.) SEEDS

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On the seeds of horse-bean (*Vicia faba* L.) many fungi are known to occur, several of them capable of infecting the young plants emerging from the seeds. They may inhibit germination and destroy the seedling. Fungi able also to infect fully developed plants are dealt in details. Organisms attacking both the underground and the aerial plant system occur among them.

Among the fungi that infect the root-system *Fusarium* species do the major damage, although under unfavourable cultivation conditions infections caused by *Macrophomina phaseolina, Pythium* spp. and *Rhizoctonia solani* may also be important. On the foliage and stem *Alternaria alternata, Ascochyta fabae, Botrytis cinerea* and *B. fabae,* as well as *Uromyces fabae,* may cause serious disease, though the importance of seed transmission of *U. fabae* is not exactly known.

In the case of pathogens with minor importance the seed transmission may primarily represent a quarantine problem, regarding their introduction in new areas. The role of organisms which damage the plants only accidentally, if at all, may be important in seed storage; either in the permanent gene bank storage of seeds, or when the conditions of storage are unsatisfactory.

Keywords: fungal diseases, seed pathology, *Vicia faba*

Introduction

The horse-bean (*Vicia faba* L.) as a source of protein may fulfil an important task both in human nutrition and in animal feeding (Kurnik, 1970). Its cultivation may, however, be considerably restricted by the seed-borne diseases. Many fungi are known to infect its seeds, and are listed together with the major literary sources in Table 1. Yet, on the pathogenity of fungi occurring on the seeds, data are not always available. So much can, however, be stated that the seeds may be damaged not only by fungi attacking the adult plants, too, but also by those which cause deterioration of quality in the stored seeds (Neergaard, 1979).

The paper deals especially with fungi known to be pathogenic, since a considerable proportion of them are known in Hungary from other host plants (Ubrizsy, 1965), however on horse-bean seeds only few Hungarian publications have appeared (Halmágyi, 1984; Lenti, 1986, 1990; Simay, 1986a, 1986b, 1987a, 1987b, 1990).

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Table 1

Fungi published from Vicia faba L. seeds

Fungi	References
1. *ZYGOMYCETES*	
Absidia sp.	79
Mucor sp.	1, 78, 79
Mucor hiemalis	1, 2, 98
Mucor racemosus	1, 98
Rhizopus sp.	78
Rhizopus microsporus	98
Rhizopus stolonifer	2, 79, 96
2. *OOMYCETES*	
Peronospora viciae	78
Pythium debaryanum	78
3. *ASCOMYCETES*	
Chaetomium sp.	79
Erysiphe communis	78
Mycosphaerella pinodes	78
Peziza ostracoderma	79
Sclerotinia sclerotiorum	78
Sclerotinia trifoliorum	95
Sordaria fimicola	79
4. *BASIDIOMYCETES*	
Uromyces fabae	70, 78
5. *DEUTEROMYCETES*	
Acremonium sp.	79
Alternaria sp.	41, 87
Alternaria alternata	58, 78, 79, 89, 95, 98
Arthrinium phaeospermum	79
Ascochyta boltshauseri	78
Ascochyta fabae	3, 58, 70, 75, 78, 79, 87, 95
Ascochyta pisi	78
Ascochyta viciae	78
Aspergillus sp.	1, 41, 58, 78, 79, 95
Aspergillus flavus	1, 2, 86
Aspergillus nidulans	2
Aspergillus niger	1, 2, 58, 86, 96
Aspergillus ochraceus	98
Aspergillus phoenicis	98
Aspergillus repens	98
Aspergillus terreus	2
Aspergillus versicolor	98
Aspergillus wentii	98
Aureobasidium pullulans	79
Bipolaris sorokiana	95
Bipolaris spicifera	1, 22, 86, 95
Botrytis cinerea	3, 78, 79, 87, 90, 95, 98
Botrytis fabae	3, 70, 75, 78, 79, 87, 90, 95
Cercospora fabae	78
Cercospora zonata	78
Chrysosporium pannorum	79
Cladosporium sp.	79
Cladosporium cladosporioides	96, 98
Cladosporium herbarum	78, 96
Colletotrichum dematium f. truncata	95
Colletotrichum lindemuthianum	78

Table 1

Cont.

Fungi	References
Colletotrichum villosum	75, 78
Curvularia lunata	96
Drechslera sp.	1, 58
Epicoccum purpurascens	79
Fusarium sp.	41, 58, 70, 78, 87
Fusarium acuminatum	98
Fusarium avenaceum	78, 79, 95, 98
Fusarium culmorum	79, 95
Fusarium equiseti	95
Fusarium graminearum	79
Fusarium moniliforme	1, 2
Fusarium oxysporum	1, 3, 63, 75, 78, 88, 95, 98
Fusarium pallidoroseum	79, 95
Fusarium poae	79, 95
Fusarium sambucium	79
Fusarium solani	75, 86, 95
Fusidium sp.	79
Gliocladium sp.	79
Gliocladium catenulatum	87
Harzia acremonioides	79
Kabatiella nigricans	78
Macrophomina phaseolina	58
Paecilomyces sp.	79
Penicillium sp.	1, 58, 79
Penicillium chrysogenum	2
Penicillium citrinum	1, 2
Penicillium funiculosum	2
Penicillium hordei	98
Penicillium lanosum	98
Penicillium pinophylum	86
Penicillium verrucosum	98
Phoma sp.	58, 79
Phoma eupyrena	79
Phoma herbarum	79
Phoma pinodella	95
Phomopsis phaseoli	95
Phyllosticta viciae	78
Rhizoctonia sp.	79
Rhizoctonia solani	75
Scopulariopsis brevicaulis	79
Stemphylium botryosum	38, 70, 95, 98
Trichocladium asperum	39
Trichoderma viride	79
Trichotecium roseum	24, 67, 79, 87, 95, 98
Ulocladium atrum	98
Ulocladium consortiale	10, 38, 79
Verticillium tenerum	79

Pathogenic fungi occurring on horse-bean seeds

Peronospora viciae (Berk.) De Bary: Data on the occurrence of horse-bean disease caused by *Peronospora* are only sporadically occurring (Blaeser-Diekmann, 1982; Jamoussi, 1968; Marras, 1963; Sávulescu, 1948), though the pathogen is wide-spread on other legumes (Mukerji, 1975). Its occurrence in Hungary is mentioned by Podhradszky (1960). On the importance of its transmission by seed no data are available, though this is hinted at in the work of Rádulescu and Negru (1971) and Jamoussi (1968) described its occurrence on the horse-bean pod.

Pythium debaryanum (Hesse) Hesse: The species was reported from horse-bean in several cases, but in some cases other species were also identified as *P. debaryanum* (Domsch et al., 1980). According to our data the pathogenity of 10 *Pythium* species for *V. faba* plants is known (Schultz, 1943, 1950; Sen and Lal, 1969; Takahashi and Morimoto, 1954; Yamamoto and Maeda, 1961), but the epidemic role of seed transmission in this genus of fungi has not been established.

Erysiphe communis (Wallr.) Link: The occurrence of this fungus on horse-bean seeds is mentioned only by Rádulescu and Negru (1971). Csorba and Berend (1965) likewise reported the occurrence of another species — *E. pisi* De Candolle — on *Vicia* species, and discussed *E. communis* as a pathogen specialized for other plants. Thus, the fungus mentioned by the Roumanian authors can also probably be regarded as *E. pisi*.

Mycosphaerella pinodes (Berk. et Brooks) Vestegr.: *M. pinodes* is a frequent pathogen on various legumes (Holliday and Punithalingam, 1972a). Its seed transmission plays an important role in spreading the disease in the case of several plants, mainly peas (*Pisum sativum* L.) (Neergaard, 1979; Vörös and Husz, 1965). Podhradszky (1960) described it in Hungary on horse-bean, but its transmission by horse-bean seed is only mentioned by Rádulescu and Negru (1971).

Sclerotinia spp.: Of the *Sclerotinia* species two are known as pathogens of horse-bean, and they also occur in Hungary (Csorba and Berend, 1965). *Sclerotinia sclerotiorum* (Lib.) De Bary is a polyphagous pathogen, and according to Gindrat (1969) this fungus is one of the most important fungi on horse-bean, too. Jellis, Smith and Scott (1990) found primarily attacking horse-beans sown in spring, while on horse-beans sown in autumn another species — *S. trifoliorum* Eriksson — occurs more frequently. The latter has only recently become known for seed transmission in Hungary (Simay, 1990), though from various leguminous plants it was known earlier (Csorba and Berend, 1965). Since in the case of *S. sclerotiorum* the infected seed is the primary source of disease for some plants (Agarwal and Sinclair, 1987; Neergaard, 1979), the importance of primary infection caused by the infected seed cannot be neglected in the case of horse-bean (Anonymous, 1965a). The infection of seed by *S. trifoliorum* is also known in several plants (Frandsen, 1946; Neergaard, 1979), mainly legumes. Its importance for horse-bean is less known.

Uromyces fabae (Persoon) De Bary: The rust disease of horse-bean occurs almost everywhere it grows. Despite this, the importance of its transmission by seed is not yet determined. Notwithstanding the severe infection observed, Lelley (1964) does not mention its seed transmission. In the case of other *Uromyces* species, and

of *U. fabae* in lentil (*Lens culinaris* Medik.) the sporae contaminating the seed are known to cause diseases (Agarwal and Sinclair, 1987; Emdal and Foldo, 1979; Neergaard, 1979).

Alternaria alternata (Fr.) Keissler: Many *Alternaria* species are known to damage various plants, and one of them — *A. alternata* — is described in the pathological literature as having a large number of host plants, and is also very frequently isolated from other substrates (Domsch et al., 1980). The first serious disease in horse-bean was reported by Ibrahim and Michail (1968), and on the bases of the increasing number of descriptions published since then, *Alternaria* has become one of the most important diseases of horse-bean (Furgal-Wegrzycka, 1984; Sumar et al., 1982). Damages caused by it in Hungary have been known since 1985 (Simay, 1987a).

Ascochyta spp.: According to literary data four *Ascochyta* species may cause infections on horse-beans (Neergaard, 1979; Podhradszky, 1960; Rádulescu and Negru, 1979; Simay, 1986a, 1990; Sumar and Howard, 1983). Of these species, *A. boltshauseri* Saccardo was originally described as a pathogen of beans (*Phaseolus vulgaris* L.). Its occurrence on horse-bean *(V. faba)* has been mentioned by several authors; Békési (1965) reported it from Hungary. The symptoms, morphological data and host-range (only horse-bean was successfully infected by the fungus isolated by him) he described do not essentially differ from the characteristics of *A. fabae* Speg. The two are therefore supposed to be identical, and the author observed, in fact, the occurrence of *A. fabae*. Similar results are published by Tikhonova and Kashmanova (1970). These data suggest that the *A. boltshauseri* occurrences on horse-bean known from the literature, or at least a large part of them, actually refer to *A. fabae*.

In the case of *A. pisi* Lib., a similar reference is made to its infecting the horse-bean and other *Vicia* species (Holliday and Punithalingam, 1972b). Its transmission by horse-bean seed is mentioned by Rádulescu and Negru (1971), and there even are data on its survival in horse-bean seed for years (Agarwal and Sinclair, 1987). On spontaneous infection of horse-bean by *A. pisi* there are but few data available (Bikmukhametova, 1963; Mujica, 1943; Podhradszky, 1960; Sprague, 1929; Tikhonova and Kashmanova, 1970). Therefore, the assessment of its importance requires further investigations, likewise with *A. viciae* Lib., whose occurrence on horse-bean has only been reported by Rádulescu and Negru (1971).

A pathogen of *V. faba* widely known to be important is *A. fabae* Spegazzini which is encountered in almost every place where horse-bean is grown (Gaunt, 1983). Its occurrence in Hungary is equally known from field infection and seed transmission (Lenti, 1988; Simay, 1986a, 1988, 1990). As this pathogen is not always able to hibernate outdoors (Wallen and Galway, 1977), infected seed lots are the main sources of infection. From infected seedlings the disease can quickly spread, and according to Hewett (1973) a 2—15% initial infection may cause 76—79% infection by the time of harvesting.

Bipolaris spp.: Both *Bipolaris* species known from horse-bean seeds (Simay, 1990) are known in Hungary from field infection (Simay, 1988b), too. Of the two species *B. sorokiana* (Sacc.) Shoemaker was earlier observed in Hungary primarily

on monocotyledonous plants (Bánhegyi et al., 1985). In the foreign literature, Ruokola and Vestberg (1978) mention field infection, noting leaf spottedness caused by *B. sorokiana*. *B. spicifera* was earlier described from similarly few places. Outside Hungary its infection in horse-bean is known from Iraq and Sudan (El Nur and Freigoun, 1970; Sharif et al., 1987). Transmission of either of the two species by seed has been established only in a few places outside Hungary (Chidambaram et al., 1973; Abdel-Hafez, 1984, 1988; Khan et al., 1984).

Botrytis spp.: The two *Botrytis* species occurring in horse-bean seeds, *B. cinerea* Persoon and *B. fabae* Sard., cause the same disease: horse-bean chocolate spots, which besides ascochytosis is the most important disease of horse-bean (Gaunt, 1983). The disease is known in Hungary where both pathogens could be detected (Simay, 1987b). *B. cinerea* is a polyphagous pathogen which, as a weak parasite, may cause disease among many plants (Vörös and Husz, 1965). To its role in the disease developing in horse-bean, attention was called by Harrison (1984), though it had been known earlier from this host plant (Blotnicka, 1979; Cadene et al., 1970; Sundheim, 1973), and horse-beans can also be successfully infected with isolates obtained from other plants (Kovács and Tüske, 1983; Enisz and Kredics, 1986; Sundheim, 1973). The transmission of *B. cinerea* by seeds of various plants is known (Neergaard, 1979), but fungal organs hibernating in plant remnants may also be initial sources of the disease, since they are able to survive in the soil for a long time (Domsch et al., 1980).

B. fabae only infects leguminous plants, according to our knowledge, and widely occurs in the growing areas of horse-bean (Gaunt, 1983). The symptoms caused by *B. fabae* are also chocolate spots, but they can be more intensive than those caused by *B. cinerea* (Sundheim, 1973). In Hungary it is known from *Phaseolus* and *Vigna* species, outside horse-bean (Tóth and Zahorecz, 1970). Its transmission by seed is only known in the case of leguminous crops (Neergaard, 1979), too, and the seed transmission is of primary importance when fresh seeds are sown, because the storage considerably decreases the efficiency of pathogen transmission (Harrison, 1976). Its overwintering on horse-bean stalks is also proved (Harrison, 1979).

Cercospora spp.: The occurrence of three *Cercospora* species on horse-bean is mentioned in the literature, of which *C. viciae* is only known from Venezuela (Müller and Chupp, 1942). Infections of horse-bean seeds by *Cercospora* species are described by Rádulescu and Negru (1971). The two species mentioned by them are *C. fabae* Fautr. and *C. zonata* Wint., the occurrence of which is also mentioned in the Hungarian literature (Podhradszky, 1960; Vörös and Husz, 1965), though the reports on their occurrences in other countries mainly refer to Mediterranean regions (Anonymous, 1947, 1965b; Blaeser-Diekmann, 1982; Bremer et al., 1948; Keissler, 1923; Müller and Chupp, 1942; Wheeler, 1958). On the basis of their morphological similarity and host-range, Bremer et al. (1948) consider the two fungi identical, and regard the name of *C. fabae* as a synonym of *C. zonata*.

Colletotrichum spp.: According to the literary data, three species are known from horse-bean seeds (Neergaard, 1979; Rádulescu and Negru, 1971; Simay, 1990), of which only *C. lindemuthianum* (Sacc. et Magn.) Bri. et Cav. was described on

naturally infected fully developed plants (Wallace and Wallace, 1947). In Hungary this *Colletotrichum* species is only known from French bean plants (Vörös and Husz, 1965). The other species known in Hungary are *C. dematium* (Pers. ex Fr.) Grove f. *truncata* (Schw.) Arx, which was also observed on horse-bean seeds (Simay, 1990). Although the seed transmission of various *Colletotrichum* species is a familiar phenomenon among many plants (Neergaard, 1979), the importance of seed transmission in the case of horse-bean has not been so far determined.

Fusarium spp.: The *Fusarium* species are frequent pathogens for various plants, including horse-bean. All *Fusarium* species known from horse-bean seeds are also known from other substrates in Hungary (Hornok, 1975) and several of them have been described in Hungary from horse-bean (Lenti, 1986, 1987; Simay, 1986b, 1990) too. On *V. faba* — as on other host plants — the *Fusarium* species mostly cause root rot and wilting (Salt, 1983). Among the species occurring on horse-bean seeds Singh and Singh (1986) found *F. oxysporum* Schlecht to have major importance, as this fungus causes the greatest damage horse-bean together with *F. avenaceum* (Fr.) Saccardo and *F. solani* (Mart.) Saccardo (Aréstegni and Dongo, 1974; Blotnicka, 1979; Salt, 1983; Vinitskaya, 1963 etc.). These three species are even known to be specified for horse-bean as their host plant (Yu, 1944; Yu and Fang, 1948).

The occurrence on horse-bean plants of the other *Fusarium* species described from seeds is known from the works of Bikmukhametova (1963), Boughey (1942), Clarkson (1978), Harrison (1981), Ruokola and Vestberg (1978), Salt (1983), Taylor and Parkinson (1961), Yamamoto et al. (1955). In the case of *F. moniliforme* Sheld., a certain host specificity can also be assumed, as Boughey (1942) reported considerable damages done by this fungus, while Youssef and Mankarios (1974) observed the growth stimulation of horse-bean seedlings in the presence of this fungus.

Macrophomina phaseolina (Tassi) Goidanich: *M. phaseolina* is a polyphagous root parasite (Holliday and Punithalingam, 1970). In Hungary it has been recognized on horse-bean since 1985 (Simay, 1987c). Despite its wide distribution and polyphagous nature, few data are available on its occurrence on horse-bean (Anonymous, 1955; Goidanich and Camici, 1947; Nafal et al., 1985; Simay, 1987c). Its transmission by seed is known among various plants, including horse-bean (Agarwal and Sinclair, 1987; Khan et al., 1984; Neergaard, 1979), but its epidemiological importance in the case of horse-bean remains uncertain.

Rhizoctonia solani Kühn: *R. solani* — like the former fungus — is a polyphagous pathogen also known for a large number of host plants in Hungary (Vörös and Husz, 1965). Salt (1983) reported the occurrence of this pathogen on horse-bean in Hungary, but the publication to which he refers (Salem et al., 1977), though edited in Hungary, does not mention this occurrence. Foreign data concerning the importance of the fungus are also inconsistent. Gindrat (1969) mentions it as one of the most important pathogens, while according to Kovácikova (1977) its occurrence is incidental. Its transmission by horse-bean seed is important when it concerns its introduction in new areas (Jauch, 1947).

Phoma pinodella (L. K. Jones) Morgan–Jones et Burch: *P. pinodella* attacks almost all aerial parts of various leguminous plants (Punithalingam and Gibson,

1976; Vörös and Husz, 1965). As for horse-bean, it is similarly known from various plant parts (Bremer, 1944; Hanounik and Maliha, 1983), but its transmission by seed is only mentioned by Simay (1990) for this host. Hence, the importance of seed transmission requires further investigations to be cleared.

Phomopsis phaseoli (Desm.) Saccardo: *P. phaseoli* is known from numerous host-plants including many legumes (Cerkauskas et al., 1980; Hepperly et al., 1980; Kulik, 1984 etc.), but in regard to horse-bean it has only been observed in Peru (Bazon de Segura, 1945) and Hungary (Simay, 1990). When artificially infected the horse-bean often remains symptomless (Kulik, 1984), so the pathogenic nature of the fungus cannot be reliably established.

Stemphylium botryosum Wallr.: *S. botryosum* does not belong to those pathogens causing serious diseases to horse-bean, although throughout the horse-bean areas of the world it is frequently encountered (Gaunt, 1983). It is a polyphagous fungus or a weak parasite found often on necrotized organic matter, straw, or even in the soil (Domsch et al., 1980; Corlett et al., 1982; Vörös and Husz, 1965). From field infections of horse-bean it has been recognized in Hungary since 1987 (Simay, 1988a). It is known to be transmitted by seed to many plants (Neergaard, 1979), and occurs commonly in *V. faba*. Through natural infection it mainly causes leaf spots (Mansour, 1980; Ruokola and Vestberg, 1978; Simay, 1988a; Teuteberg, 1980), though it may also infect other plant parts (Filipowitz, 1984).

Trichotecium roseum Link: This fungus is a saprophyte rather than a typical pathogenic organism and belongs to the common fungi of soils (Domsch et al., 1980). However, when settled on the seeds it may do considerable damage to them (Conners, 1976; Lenti, 1990; Neergaard, 1979; Simay, 1986a, 1990). The filtrate of its culture is also able to inhibit germination (Desai and Siddaramaiah, 1980). According to Lenti (1990) seed infection caused by *T. roseum* may be one of the causes of seed destruction in the sowing bed.

Ulocladium spp.: Of the two *Ulocladium* species observed on horse-bean seed *U. atrum* Preuss is not known to occur on fully developed plants. For *U. consortiale* (Thüm.) Simmons Barbu and Dinescu (1970) mention leaf- and pod infections besides seed transmission. No data are, however, available on the importance of either of the two species in horse-bean cultivation.

Other fungi: We possess no data on the occurrence of the other fungi listed in Table 1 on horse-bean in Hungary, and on their importance in cultivation. Yet, fungi not always causing diseases to fully developed plants may still represent a problem when are the seeds storing (Neergaard, 1979). They may impair the germination ability and practical value of the seeds; this effect becomes stronger when the conditions of storage are unsatisfactory (Halloin, 1986). Studying the pathological correlation between storage humidity and storability, Carontino et al. (1976) point out the favourable effect of high degrees of humidity on the multiplication of certain fungi. Mikhailenko (1965) is of the opinion that certain long-action dressing agents can be applied to stored seeds as well.

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Obituary

ERNEST ROBERT SEARS (1910—1991)

Ernie Sears, the most famous professor of the University of Missouri, passed away February 15, 1991. He was a geneticist par excellence. Through his pioneering and classical work polyploid species of plants, particularly wheat and relatives, became important genetic organisms. His theoretical basic work made impact on research far beyond wheat, and has affected the development of even human cytogenetics. Ernie was a fortunate person also because he lived long enough to see the application of his discoveries to practical agriculture. Plant breeding endeavors based on his theoretical work, and the genetic stocks developed by his laboratory bring millions of dollars of return year after year throughout the world.

Dr. Sears published well over 100 scientific papers, and was working on a wheat cytogenetics monograph that he could not complete. All his major accomplishments cannot be surveyed here. He has developed the complete monosomic and nullisomic series of hexaploid wheat that enable investigators to analyze the total gene content of each individual chromosome of this species. With the cooperation of a graduate student (M. Okamoto) they discovered a gene in chromosome 5B of wheat that suppresses pairing between the homologous series of chromosomes. This discovery made then possible to transfer any length of desirable genetic sequences from wild relatives of wheat to cultivated varieties. Because of the results of this research, chromosomal engineering was born. Ernie (with the cooperation of E. S. McFadden) was the first to identify the origin of the D genome of wheat through the cytogenetic analysis of a colchicine-doubled hybrid of *Triticum dicoccoides* and *Aegilops squarrosa* (current name *T. tauschii*). These hybrids represented a first synthesis of a hexaploid wheat, *T. spelta*. The results were also important milestones in experimental evolution of plants. Then he went on and identified the relationship among each of the individual chromosomes of the three genomes present in hexaploid wheat. For this purpose he used the nullisomic-tetrasomic compensation method. Homologous chromosomes in four doses more or less compensated for the chromosomes absent in the nullisomic plants whereas tetrasomy for non-homologous chromosomes aggravated the problems of the nullisomics, detectable by morphological observations. He has developed methods for mapping genes and centromeres in allopolyploids by using the nullisomic and telosomic series of chromosomes he has generated. He has also shown that telosomes can be readily obtained through misdivision of univalents and generated telosomic and double

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telosomic series which permitted unique insight into the organization and gene content of wheat chromosomes and chromosome arms. Dr. Sears made also fundamental contributions to the nature of mutation in alloploid species such as wheat. He has discovered that generally recessive mutations are expressed in hexaploid wheat only when they are hemizygous-ineffective, i.e. the mutations are active, and represent recessive suppressors. Other type of mutations are not expressed because the homologous counterparts effectively conceal the recessive defects.

Ernie was a real virtuoso of cytogenetics. He could readily count chromosomes and sometimes even identify the genetic constitution of a meiocyte in squash preparation without the use of a coverslip. He was a most meticulous worker who organized his material the most efficient way, and always recognized the most basic features of his observations by logical means. He was great at his techniques but first of all he was a really creative scientist. He never forgot the precepts of the late Harvard Professor Louis Agassiz: it is more dignified to think it out than to work it out. Of course, after he thought it out, he has worked it out by sets of interlocking evidences. Research gave him great pleasure and satisfaction and did not consider it work. Even after formal retirement he went into his small laboratory before 7 a.m. each morning, and I commonly found him in his office or in the green house during weekends or on holidays when pollinations were to be made or cytological samples had to be collected. Ernie did not rely on technician's help, he did everything himself, including watering his plants and sometimes filling the pots. He has conducted research to the last day of his life. One of his manuscripts is currently in press; other pieces of his research may remain unfinished. On the day of his death when I entered his laboratory, there was still the chaff on the floor that he accidentally spilled around the wastebasket as he threshed some ears the day before. Obviously, he was not feeling well, had a bad cold, otherwise he would have cleaned it up himself.

Dr. Sears maintained a very large collection of genetic stocks and supplied material to hundreds of laboratories world-wide. In the past, the seed requests came mainly from geneticists and plant breeders but he was very pleased with the increasing number of requests and inquiries received from molecular biologists and answered the calls with the greatest patience. He has reviewed an uncountable number of manuscripts. His editorial skills were renowned by all who were fortunate enough to get acquainted with him. Quite frequently, he had rewritten the manuscripts received and added to them his carefully worded comments, trying to use expressions as close as possible to the authors' original intentions. Sometimes, he has spent several days with a manuscript because of the linguistic deficiencies of the foreign authors, and ironed out the potential ambiguities. He was helping his colleagues even if he never met them personally or never even heard about them before. Ernie had an internal urge to make science clear and simple.

His enormous contributions to genetics were well recognized by the world. He was one of three MU faculty members ever elected to the National Academy of Sciences USA. In 1986 he shared the international Wolf Prize, second in prestige only to the Nobel Prize. He was recipient of several honorary degrees from domestic and foreign universities. Served as President of the Genetics Society of America, and

was a honorary member of the Genetics Societies of Japan and India, the American Association of Cereal Chemists, the European Wheat Aneuploid Cooperative, etc. April 19, 1974 was declared *Professor E. R. Sears Day* by the Governor of the State of Missouri. In 1989, upon Faculty nomination, he received the title of Sesquicentennial Professor. He received several most prestigious awards from the U. S. Department of Agriculture, including the highest Hoblitzelle Award. All his honors and awards are too numerous to be listed.

Besides being an extraordinary scientist, Ernie provided a role model for personal integrity, dedication to all noble causes and humanitarian principles. He has been the most unselfish and helpful person, particularly to those who most needed it. Although he had many saintly features, he was a real flesh and blood person who enjoyed all aspects of life. He has been a champion tennis and badminton player, enjoyed playing cards and good classical music. In his younger years he liked to dance regularly. He was also a very good gardener, and took care himself of the land of several acres around his home, including a small orchard and a vegetable garden. The products of this activity, fruits and vegetables, he brought each morning to the building for everybody to share.

Ernie Sears whole life was an American paradigm. He was born in a humble rural Oregon family where he learned the value of common sense, hard work and high ethical principles. He attended an one-room high school and after completing undergraduate work at the University of Oregon in Corvallis, he was accepted to Harvard University and studied there in the famous Bussey Institute under the guidance of E. M. East and Karl Sax while earned his living by washing guinea pig cages for William Castle, and worked at night as a student editor. His remarkably successful career was, however, determined solely by the brilliance of his mind and the dedication to his goals.

Professor Sears has visited Hungary on a couple of occasions, and he entertained many visitors of the country and impressed them by his generous advice and other types of assistance. It is perhaps of some interest to note that in his second paper, the first about *Triticinae*, published in 1939, among 13 references one is the citation of an early paper of Professor Barna Györffy, a most highly respected teacher of the majority of Hungarian geneticists.

Ernie is survived by his wife of 40 years, Lotti, an accomplished cytologist by her own right, and by two sons and two daughters (a perfect genetic ratio). Their daughter Barbara is also a very successful plant molecular geneticist.

The aneuploid stocks he has generated and maintained will be used for centuries to come; he has made a remarkable impact on the cultural history of mankind. Ernie has left also a not less precious legacy of humility, friendship and humanity.

G. P. RÉDEI

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Book Reviews

D. SUTIC and J. B. SINCLAIR: *Anatomy and physiology of diseased plants.* CRC Press, Inc., Boca Raton, 1991, 232 pp.

The scope of the book deals with the structural and functional changes of diseased plants. These changes are considered as pathosystems in cells, tissues and organs of plants.

Cytopathological changes, grouped to their similarities, deal with different alterations in cell structure and organization. The effect of individual cytopathological changes on development of various plant tissues is analyzed. Cytopathological and histopathological changes are described as the internal deviations in plant structure and organization. The mutual connections between the anatomical and external disease symptoms are briefly discussed in morphology of diseased plants.

The pathological changes in cell functions and tissues composed by these cells are considered in the physiology of diseased plants. In this chapter the biochemical and physiological deviations in cell membranes permeability, photosynthesis, respiration, protein synthesis, metabolism of phenolic compounds, etc., are discussed.

The final chapter of the book is concerned with various defence mechanisms against different agents of diseased plants. The cells with their own biochemical and physiological activities are described as unique carrier of plant defenses against injuries caused by pathogens or various abiotic causal agents. Conclusion: the possible genotype changes are the most efficacious weapons for plant defense.

Some new ideas and judgements reported in different chapters of this book will promote further work and developing a new knowledge on Anatomy and Physiology of Diseased Plants. The book is of wide interest to phytopathologists, botanists, physiologists and agriculturalists.

MÁRIA V. NÉMETH

R. HAMPTON, E. BALL and S. DE BOER (Eds.): *Serological methods for detection and identification of viral and bacterial plant pathogens.* A laboratory manual. APS Press, St. Paul, Minnesota, USA, 1990.

This book covers all aspects on serology and, as is written in the introduction, "This manual presents methods for preparing and treating antigens, polyclonal antisera, and monoclonal antibodies as well as state-of-the-art serological principles, methods, step-by-step procedures, and applications for detection and differentiation of viral and bacterial plant pathogens". The manual is divided into 5 sections and subdivided into 27 chapters written by 34 experts in the field.

In the first section the authors deal with the immune response, antigens, antibodies and antigen-antibody interactions.

The second section is subdivided into 4 chapters including preparation of antigen (A) and antibodies (B), globulin conjugation methods (C) and amplification systems (D).

The third and fourth sections deal with serological techniques: single and double diffusion, microprecipitation, immunoelectrophoresis, rocket immunoelectrophoresis, enzyme-linked immunosorbent assay (ELISA), radio-immunoassay (RIA), enzyme-linked fluorescent assay (ELFA), dot immunobinding assay (DIBA), western blotting, immunofluorescence, latex agglutination, serological specific electron microscopy (SSEM).

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The fifth section presents new and special topics, such as immuno-isolation (bacteria), infectivity neutralization, translation product immunoprecipitation and immunological techniques for molecular plant pathology.

Each chapter consists of principle, materials, procedures, comments or applications and literature citations.

The text is well-written and illustrated, and easy to understand. The manual closes with a glossary of terms, sources of supply, index of general, index of bacteria, index of chemicals and index of viruses.

This laboratory manual is recommended to all students and researchers studying detection and identification of viral and bacterial plant pathogens.

I. TÓBIÁS

H. GELDERMANN and F. ELLENDORFF: (Eds.): *Genome analysis in domestic animals*. Weinheim (Germany), New York, Basel, Cambridge, 1990.

This publication is the summary of lectures delivered at a symposium in Hannover (1990) on Genome analysis (analysis of genes localized in haploid chromosome complements, an indispensable condition of normal gamete and zygote formation). This is an essential condition of clarifying and understanding the bases of the molecular biology. It is particularly important in developing an up-to-date biotechnology in livestock breeding, in order to improve the quality parameters. The book deals with the analysis and function of genes determining the different characteristics. A further task of the work is to promote development with the view of a cooperation of the various disciplines. These objectives throw up important questions of principle, which is particularly remarkable in the modern biotechnical research when one must be aware of the possibilities and risks.

The authors hope that the examples listed in the book will convince the reader that by combining mathematical and physical methods, using computer facilities, the possibilities of solving important biological questions can be widened. Improved DNA technics may render it possible to make use of the large number of genetic markers with the help of an analysis of the linkage genes. Accordingly, biotechnology can play an important role in improving the future animal populations. The molecular genetics, the recombination and polymorphous DNA regions, and the genetic maps, which may give information for the fundamental genome analysis may be of help. The new methods may usefully complement the expression of genes in the course of selection, and the character of the polygenic properties (e.g. the quality of lactalbumen, etc.).

A. HORN

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Plant physiology and agrochemistry

OCCURRENCE AND PHYSIOLOGICAL ROLE OF BENZOXAZINONES AND THEIR DERIVATIVES. IV. ISOLATION OF HYDROXAMIC ACIDS FROM WHEAT AND RYE ROOT SECRETIONS

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Roots of young seedlings of wheat and rye secrete cyclic hydroxamic acids (benzoxazinones). Secretion of hydroxamic acids by wheat roots in an Fe-free medium increases as the nutrients stored in the endosperm are depleted, which effect, however, is repressed by Fe(III)-ions added to the nutrient solution. Fe-deficiency entails increased root growth, which will also decrease if iron is applied.

Hydroxamic acid secretion by rye roots is increased by Fe(III)-ions added to the nutrient solution and Fe-chlorotic symptoms are moderated. Cyclic hydroxamic acids probably play a role in the Fe-uptake (metabolism) of cereals.

Keywords: BOA, cyclic hydroxamic acids, DIBOA, DIMBOA, Fe-uptake by wheat and rye, phytosiderophores

Introduction

Roots of grasses secrete several chemical compounds (organic acids, amino acids, phytosiderophores, etc.) some of which play roles in the uptake of ions. Members of the mugineic acid family, which have a phytosiderophore function, deserve attention (Kawai et al., 1988). That is, they assist the uptake of iron and other microelements (Römheld and Marschner, 1986). The case is that grasses have a specific Fe-uptake mechanism (Marschner et al., 1986); the phytosiderophores selected by the roots form complexes with the Fe(III)-ions of the soil. Grass roots absorb otherwise insoluble Fe(III)-ions in the form of such complexes, whereas other plants reduce them outside the plasmalemma and it is together with the secreted compounds that the reduced iron enters the cytoplasm.

Barley, wheat, oats and rice secrete considerable amounts of compounds of phytosiderophore activity, while maize secretes only a limited amount of 2'-deoximugineic acid (Kawai et al., 1988). Our earlier experiments (Pethő, 1992b) have

Abbreviations
BOA: benzoxazolinone
MBOA: 6-methoxy-2-benzoxazolinone
DIBOA: 2,4-dihydroxy-2H-1,4-benzoxazin-3(4H)-one
DIMBOA: 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one

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revealed that maize roots secrete 7-methoxy-benzoxazinone, a cyclic hydroxamic acid. In the case of plants grown on nutrient solutions, the hydroxamate content of their roots and the amount of secreted hydroxamate depend on Fe-supply. It was supposed the cyclic hydroxamic acid in the maize, just like the hydroxamate-type siderophores in microorganisms, plays a role in the iron-uptake of maize or perhaps that of other grasses as well.

Later we attempted to detect these compounds in root secretions of other plants containing cyclic hydroxamic acids. It was Wahlroos and Virtanen (1959) who isolated cyclic hydroxamic acids from wheat, and Virtanen and Hietala (1960) managed to do the same with rye.

In the shoots of rye it is 2,4-dihydroxy-2H-1,4-benzoxazin-3(4H)-one (DIBOA) and in those of wheat it is 2,4-dihydroxy-7-metoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA) that can be found in larger amounts (Tang et al., 1975). The roots of both plants contain only traces of DIBOA and rather more DIMBOA. In the chain of biosynthesis of these compounds (Niemeyer, 1988) metoxy-derivatives appeared only later and can be considered derivatives of the demetoxy form.

The biological role of cyclic hydroxamic acids is diverse but currently it is their role in the defence mechanism that is mainly emphasized (Niemeyer, 1988). The fact that they form complexes with Fe(III)- and Cu-ions (Tipton and Buell, 1970; Hiriart et al., 1985) makes their roles in ion-uptake and transfer plausible, but remains to be identified precisely (Pethő, 1992b).

Materials and methods

The experiments were carried out with wheat (*Triticum aestivum* L. cv. "GK Zombor") and rye (Secale cereale L. cv. "Dankowszkie nova") plants grown on a nutrient solution (Römheld and Marschner, 1986). The germinating grains were placed on plastic nets on top of dark one-litre pots filled with ion-free water. In order to provide the necessary humidity, the first two days of growth occurred in a closed environment. A week later, water was replaced by a nutrient solution which was changed every three days, and aeration was also continuous.

The plants were precultured under controlled climatic conditions (day/night regime of 14/10 h at 25/22 °C, relative humidity 70–75%, light intensity of 2600 lx provided by fluorescent tubes).

After 10–14 days the plants were removed from the nutrient solution and put in distilled water from 2 to 6 hours following the start of the light period.

The materials secreted by the roots in the four-hour period were extracted by ethyl-acetate, following the reduction of the volume of root washings in vacuum. The organic phase was evaporated to dryness and then separated on Whatman 3MM paper by ethyl acetate—formic-acid—water (60 : 5 : 35) solvent. Materials from the Rf = 0.8–1.0 region were eluted by methanol. The residues which had been evaporated to dryness were later dissolved in 60% methanol containing 0.1 mol acetate-buffer (pH = 3.5). Twenty microliters of this solution was analysed using a 25 cm Chromsil C_{18} column in a Labor MIM Liquochrom equipment. Elution was with 50% methanol containing 0.02 mol acetate buffer (pH = 5.6), or with a linear gradient of methanol (20 to 50%) generated by a solvent programmer and two pumps. The flow rate was 1.0 mL/min and detection was done at different wavelengths.

The HPLC analyses of the samples were basically done according to the method by Lyons et al. (1988). In the case of each experiment we indicated whether the elution was done in a 50% methanol or in a linearly increasing methanol concentration. In order to identify the compounds, apart from retention time, we used the colour reaction of $FeCl_3$ with cyclic hydroxamic acids and heat treatment. In the latter case, benzoxazinones when heated (especially in an alkaline medium) will form into respective benzoxazolinones; that is, DIMBOA and DIBOA will become MBOA and BOA, respectively.

Hydroxamic acids in a diluted aqueous medium—especially while the root secretions are concentrated—will decompose easily (Brendenberg et al., 1962) and, following a quick aldol-formation, several transitory products result. The absorption of intermediaries and their chromatographic behaviour will differ, which makes their quantitative identification under such conditions difficult. Therefore we thought it more advantageous to make hydroxamic acids into benzoxazolinones, since they have a good HPLC elution, have specific absorption maximums of their own, and the products (MBOA and BOA) of the two hydroxamic acids (DIMBOA and DIBOA) can be well separated. Depending on the experimental conditions, 40–75% of DIMBOA will form into MBOA (Woodward et al., 1978). Our experiments (Pethő, 1992a) show that transformation in an ammoniac medium at 60–70 °C is fast and relatively quantitative and other products can only be found in traces.

The isolation of standards was done from maize and rye according to the method by Corcuera et al. (1978). Following an ethyl acetatic extraction, a paper-chromatographic purification was first carried out. The $FeCl_3$-positive region underwent elution and was further purified on a Sephadex LH-20 column. The quantitative determination was done on the basis of a calibration curve prepared from crystalline compounds. The data are from a minimum of 5 experiments done at different times.

Results

Hydroxamic acids and related compounds in wheat root secretions

Materials secreted by roots of wheat seedlings in a four-hour period during the morning hours were extracted from the concentration with ethyl acetate, purified by applying paper chromatography and analysed in a liquid chromatograph. It was stated that DIBOA was present in larger amounts in root secretions (Fig. 1). In the process of a linear gradient elution, the peak at 10.1 minutes was the same as the DIBOA isolated from rye, while the compound of about 13 minutes corresponded to the MBOA resulting from the decomposition of 7-methoxy-benzoxazinones.

Benzoxazinones will decompose in the process of preparation and the presence of corresponding benzoxazolinones can be shown on the chromatogrammes. When the sample was kept at a temperature of 70 °C with 0.1 mol NH_4OH applied for 10 minutes, the peak at about 13 min increased considerably at 290 nm, whereas the material present at 11.4 minutes disappeared as a consequence of heat treatment. Since on the basis of the retention times of the standards, the former proved to be MBOA and the latter DIMBOA, it is certain that wheat root secretions also contain 7-methoxy-benzoxazinone, which when concentrated at 40 °C will already disintegrate, and the sample also contains 6-methoxy-benzoxazolinone (MBOA) (Fig. 2).

After having shown that wheat root secretions contain both 7-methoxy-benzoxazinone and its demethoxy derivative, calibration curves with purified compounds were made and the amounts of the components were determined on the bases of peak heights. These analyses were done on chromatogrammes prepared with 50% methanol. It was stated that roots of ten-day-old wheat plants contained larger amounts of DIBOA than DIMBOA (Fig. 3). Next the effects of ages and Fe-supply of wheat plants on hydroxamic acid secretion by roots were analysed. Two-week-old wheat plants did not show chlorotic symptoms and the Fe-supply did not affect the chlorophyll content of younger (second) leaves considerably. $5 \cdot 10^{-6}$ mol $FeCl_3$, applied three days before the analysis was done, raised the chlorophyll content of the second leaf blade only by 6.4% as compared to that of plants grown in an Fe-free nutrient solution.

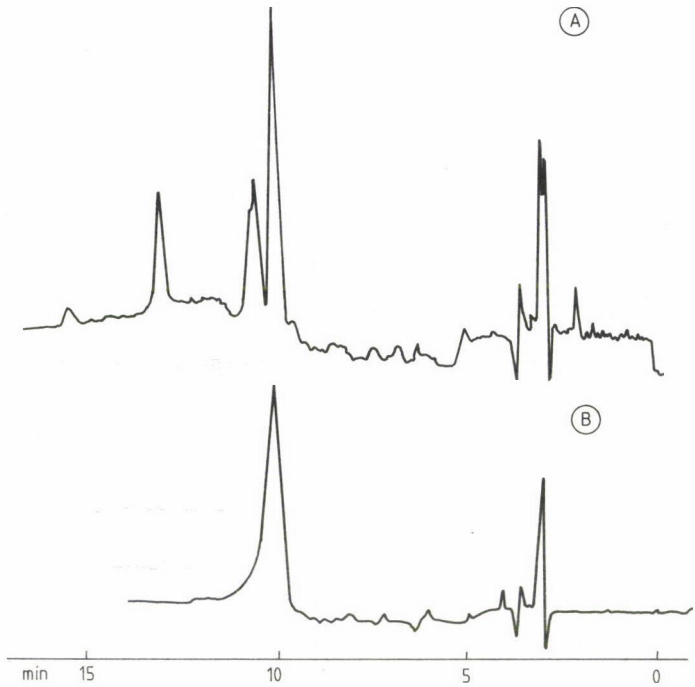

Fig. 1. Identification of DIBOA in wheat root secretions on the basis of retention time. A: exudate of wheat; B: DIBOA standard. Methanol concentration was linearly increased from 20% to 50%. Detection at 290 nm

It was also stated that (Table 1), with the depletion of nutrients and iron stored in the endosperm, the amount of hydroxamic acid secreted by the roots increased. When $FeCl_3$ was added to the medium on the 10th day, root secretions of fourteen-day-old plants showed a considerable decrease of hydroxamic acids as compared to the Fe-deficient ones. According to data it is DIBOA that Fe-deficient wheat plants secrete in larger amounts. At younger ages it is DIMBOA that predominates in the exudates.

Table 1

The effect of the ages of plants and the Fe-content of the nutrient solution on the secretion of hydroxamic acids by wheat roots. The compounds were heat treated, and products of decomposition of hydroxamic acids benzoxazolinones were then analysed. Values represent the average of three samples ± the standard error of the mean

Age of plant, Fe-supply	Benzoxazolinone	6-methoxy-benzoxazolinone
	microgram/g of fresh root mass	
10 day. −Fe	0.34 ± 0.05	0.87 ± 0.09
14 day, −Fe	1.30 ± 0.12	0.52 ± 0.07
14 day, 10^{-6} mol $FeCl_3$	0.36 ± 0.04	0.16 ± 0.02
14 day, $5 \cdot 10^{-6}$ mol $FeCl_3$	0.08 ± 0.01	0.24 ± 0.02

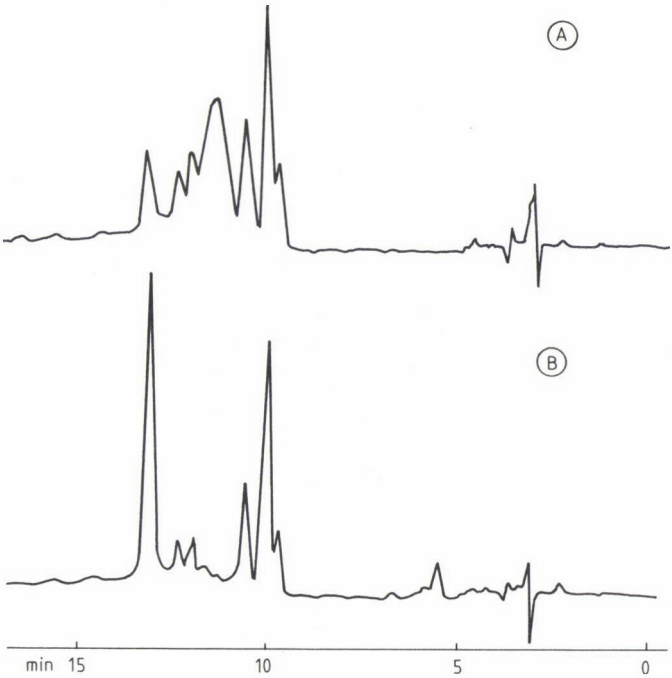

Fig. 2. Identification of DIMBOA and MBOA from wheat root exudates by heat treatment. A: control; B: heat treated sample. Detection at 290 nm, elution with increasing methanol concentration

Changes in root masses were also considered during the four days following Fe-application (Table 2). The data reveal that wheat plants respond to Fe-deficiency with intensive root growth. Fe-application either decreased or prevented root growth, or both.

Table 2

Increase in root masses of 100 wheat plants depending on Fe-supply between days 10 and 14

Treatment	Root mass growth of 100 plants	
	gram	%
Without iron	4.39	100.00
10^{-6} mol $FeCl_3$	3.72	84.75
$5 \cdot 10^{-6}$ mol $FeCl_3$	0.75	17.08

Effects of Fe-supply on hydroxamic acid secretion by rye roots

Root secretions of two-week-old rye plants were analysed first (Fig. 4). It was stated that rye secretes both DIMBOA and DIBOA, part of which, in the course of applied preparation processes, change into corresponding benzoxazolinones (MBOA and BOA).

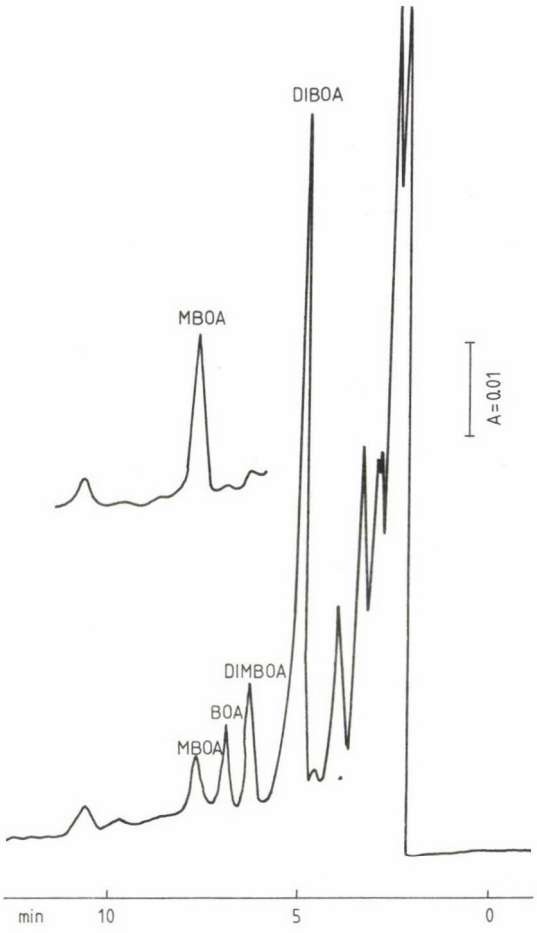

Fig. 3. Analysis of root secretions by 10-day-old wheat plants by HPCL with 50% methanol. Detection at 270 nm. INSERT the same sample detected at 290 nm. In the latter case the peak height corresponding to BOA decreased and that of MBOA increased, since the absorption maximums of the two compounds are different

While in the case of wheat plants, the Fe-free medium did not bring about chlorotic symptoms in two weeks and Fe-application hardly changed the chlorophyll content of the second leaf blade, the second leaf blade in rye was highly chlorotic on the 14th day, and Fe-application decreased chlorotic symptoms resulting from Fe-application to a large extent (Table 3). The data indicate that rye is more sensitive to Fe-deficiency, which reason prompted us to analyse the effect of Fe-supply on the secretion of hydroxamic acids. Samples in this experiment were heat treated and amounts of benzoxazolinones were measured (Table 3).

The data given in the table indicate that rye responds to Fe-deficiency in a different way than does wheat: Fe-supply will increase secretions of hydroxamic

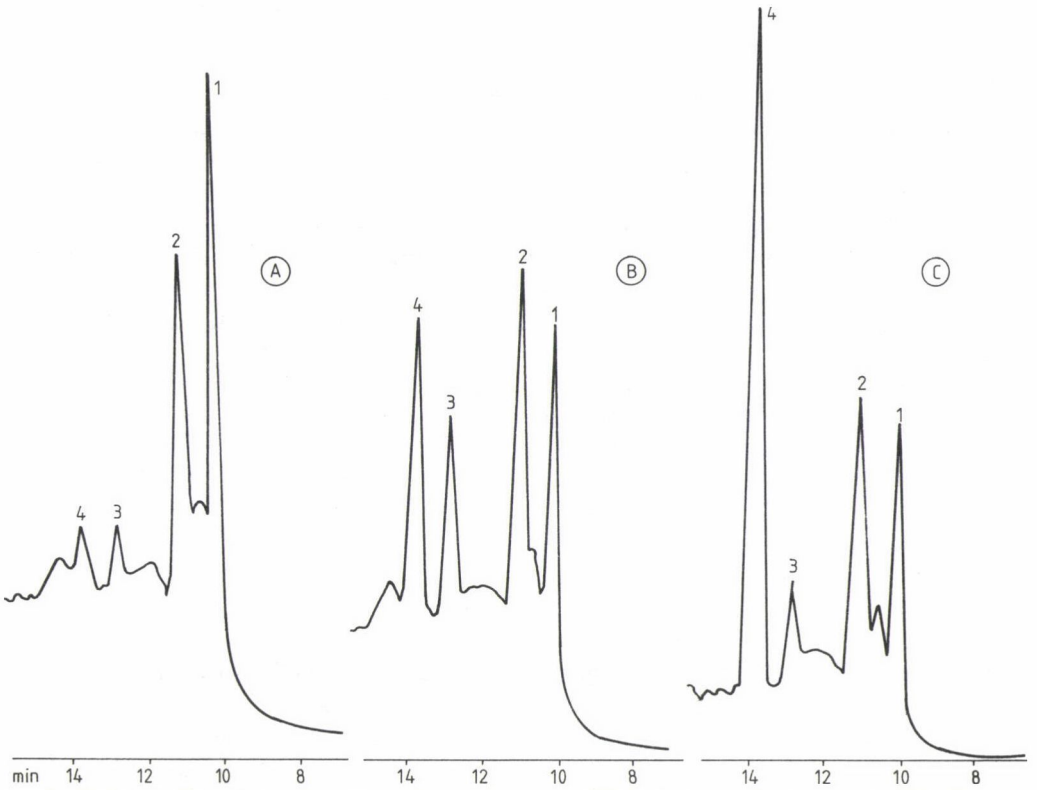

Fig. 4. Analyses of root secretions by two-week-old rye plants at different wave lengths by HPLC. Methanol concentration was linearly increased to 50%. A, B and C are detected at 254, 270 and 282 nm, respectively. 1: DIBOA; 2: DIMBOA; 3: MBOA; 4: BOA

Table 3

Effect of the iron supply of the nutrient solution on the chlorophyll content in the 2nd leaf blade and the hydroxamic acid secretion by the roots of two-week-old rye seedlings. Values represent the average of three samples ± the standard error of the mean

Iron supply	Chlorophyll a+b		BOA	MBOA
	mg/g fress leaf	%	microgram/gram fresh root	
Without iron	0.99±0.16	100.00	0.42±0.12	0.97±0.09
10^{-6} mol $FeCl_3 \cdot dm^3$	1.49±0.23	150.18	1.37±0.17	1.10±0.12
$5 \cdot 10^{-6}$ mol $FeCl_3 \cdot dm^3$	1.53±0.27	154.00	1.40±0.15	1.60±0.15

acids by rye roots. No considerable differences in the two hydroxamic acids were observed in the experiment, which is surprising since it is DIBOA that dominates in the shoots of rye, and DIMBOA can only be found in much smaller amounts.

Discussion

With the exception of extreme cases (low pH, anaerob conditions) iron is found in our soils in the form of not readily available Fe(III)-ions. Under such conditions and in Fe-free nutrient solutions, grasses will secrete phytosiderophores, which are members of the family of mugineic acids (Takagi, 1976; Takagi et al., 1986). These compounds form complexes with Fe(III)-ions in the rhizosphere, and plants take up the iron, which is essential for them, through a specific transport system (Sugiura et al., 1981). The phytosiderophore secretion in grasses is varied, e.g. maize secretes one-tenth of the amount of mugineic-type phytosiderophores secreted by barley, which is mostly studied (Kawai et al., 1988).

Our earlier experiments have shown (Pethő, 1992b) that, depending on the Fe-content of the nutrient solution, maize roots will secrete one cyclic hydroxamic acid (7-methoxy-benzoxazinone). As this compound also forms complexes with Fe(III)-ions, Tripton's and Buell's assumptions seem to be confirmed. It is likely that eyelic hydroxamic acids, similarly to hydroxamate-type siderophores of microorganisms, take part in Fe(III)-ion uptake.

This assumption also seems to be confirmed by this experiment. As this experiment shows, the two cereals, which were already known to contain benzoxazinones, will secrete these compounds through their roots under Fe-deficient conditions. There are, however, differences in the secretion of hydroxamic acids by the two plants. While in the case of wheat root, secretions of hydroxamates decreased in nutrient solutions containing Fe(III), but in the case of rye there was a condiderable increase. Wheat (Nomoto et al., 1981) and rye (Nomoto and Ofune, 1982) secrete considerable amounts of phytosiderophores of the mugineic type. Further studies are necessary to establish whether cyclic hydroxamic acids, similarly to phytosiderophores of the mugineic type, take part in the iron uptake by grasses. Features characteristic of phytosiderophores were mainly found in the process of hydroxamate secretion by wheat: Fe-deficiency entails increased secretion of hydroxamic acids and Fe-supply goes together with a considerable decrease in the secretion of this compound. Accordingly, hydroxamic acid secretion by wheat roots will respond to Fe-stress in the same way as experienced with the secretion of phytosiderophores of the mugineic type.

The increase in the secretion of hydroxamic acids by rye roots is a reminder of what was experienced in the case of maize (Pethő, 1992b). There seem to be considerable differences among plants as far as secretion of hydroxamic acids is concerned.

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EFFECT OF POTASSIUM DEFICIENCY ON METABOLISM OF CHICKPEA, *CICER ARIETINUM* L.

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The influence of potassium deficiency on some physiological processes in chickpea (*Cicer arietinum* L.) cul. chafa was investigated. K-deficiency had adverse effects on such plant growth parameters as plant height, number of leaves, leaf area and fresh and dry weights. Alterations in the macronutrient level in different plant parts in leaves were evident under conditions of potassium deficiency. K-deficient plants accumulated more Ca in leaves while other elements like Na, P, Mg, Fe, Mn and Cl were lowered. Roots of K-deficient plants showed increased levels of these elements, except Mn. In stems Ca, P and Mg levels increased while Na, Fe, Mn and Cl levels decreased due to K deficiency. These alterations were further accompanied by changes in the level of various carbohydrate and nitrogen fractions.

Keywords: *Cicer arietinum*, mineral nutrition, potassium deficiency

Introduction

Soil provides the reservoir for some fourteen mineral elements for the normal development of plants. Of these, potassium is regarded as an indispensable element for crop growth, as it is involved in every metabolic process, including activation of several key enzyme systems, carbohydrate metabolism, protein biosynthesis, assimilate translocation and stomatal movement. However, a survey of Indian soils indicates that about 20% of the soils are poor in potassium (Ghosh and Hasan, 1977). Although number of physiological diseases due to K-deficiency have been recognised in Asian countries, especially in the case of cereals like rice (Tanaka and Yoshida, 1970), little attention has been paid to potassium nutrition of legumes. Hence, the present work is devoted to the investigation of some biochemical processes in chickpea under conditions of K-deficiency.

Materials and methods

Experiment and design

Chickpea cul. chafa seeds were obtained through the courtesy of the Agricultural College, Pune. Plants were raised from healthy seeds in acid-free silica sand and supplied with Hoagland nutrient medium in plastic containers (1.0 × 0.5 × 0.5 M) with four plants in each. After 15 days of establishment, the treatment of K-deficiency was commenced following the procedure of Moore (1974). In this procedure in a K-deficient medium, K is replaced by Na and the accompanying anion is NO_3. Plants were treated with the respective nutrient media twice a week with alternate watering to avoid excess accumulation of salts and to check loss of water due to evapotranspiration. Sixty-day-old plants were subjected for analysis of such growth parameters as total height, leaf number, leaf area, fresh weight and

dry weight of the plants. Plants of the same age were harvested for analysis of various inorganic and organic constituents.

Inorganic elements were analysed from acid digest following the standard procedures described earlier (Chavan and Karadge, 1980). Magnesium was estimated following the method of Drosdoff and Nearpass (1948). The carbohydrates were estimated following the standard method of Nelson (1944) using Arseno — molybdate reagent. Total nitrogen was estimated according to the procedure of Hawk et al. (1948), while for nitrate the method of Wooley et al. (1960) was followed. Free proline and soluble proteins were determined according to Bates et al. (1973) and Lowry et al. (1951), respectively.

Each experiment was replicated at least thrice.

Results and discussion

Plant growth

The response of chickpea plants towards imposed K-deficiency is presented in Table 1. It is interesting to note here that the visual deficiency symptoms became apparent only in later phases of growth. K-deficiency adversely affected plant growth, since nearly all parameters, such as total height, root, shoot length, number of leaves, number of leaflets per leaf, average leaf area, number of branches, as well as fresh and dry weights of the plant, were considerably reduced. Similar growth stunting has also been reported by Marschner et al. (1981) in sugar beet, Repka (1983) in maize and Scherer et al. (1982) in wheat.

Table 1

Effect of potassium deficiency on some growth parameters of chickpea (Cicer arietinum)

Growth parameter	Treatment	
	Complete	Potassium deficient
Total height (cm plant^{-1})	36.52 ± 2.27	27.73 ± 3.63
Shoot length (cm plant^{-1})	25.14 ± 2.23	19.66 ± 2.05
Root length (cm plant^{-1})	11.38 ± 1.56	8.07 ± 1.86
Total leaves (plant^{-1})	83.70 ± 13.40	71.40 ± 12.20
Leaflets/leaf	11.00 ± 0.32	9.00 ± 0.63
Average leaf area (cm^2)	1.44 ± 0.92	0.92 ± 0.38
Number of branches (plant^{-1})	8.00 ± 2.63	4.00 ± 0.84
Fresh weight (g plant^{-1})	20.904 ± 2.346	14.764 ± 3.780
Dry weight (g plant^{-1})	5.735 ± 1.006	3.441 ± 0.982

Mineral nutrition

The effect of K-deficiency on mineral status is presented in Table 2. It is well evident from the table that, due to K-deprivation, the K status of the plant is lowered in all plant parts. The same table designates that sodium level is lowered in leaf and stem tissue. However, Na content is almost doubled in root tissue of K-deficient plants. Though Na^+ has been reported to act as substitute for K^+ in

Table 2

Effect of potassium deficiency on inorganic constituents in different parts of chickpea (Cicer arietinum)

Plant Part	Treatment	Na^+	K^+	Ca^{2+}	P^{5+}	Mg^{2+}	Fe^{2+}	Mn^{2+}	Cl^-
		(Meq g^{-1} Dry tissue)							
Leaf	Complete	0.068	0.075	3.044	0.036	0.280	0.179	0.056	0.635
	Potassium deficient	0.047	0.016	3.543	0.035	0.255	0.122	0.051	0.139
Stem	Complete	0.054	0.076	0.798	0.033	0.292	0.093	0.051	0.486
	Potassium deficient	0.049	0.007	1.347	0.036	0.304	0.057	0.050	0.114
Root	Complete	0.021	0.016	0.350	0.026	0.170	0.028	0.131	0.224
	Potassium deficient	0.050	0.005	0.299	0.046	0.455	0.047	0.123	0.472

Data represent average of three determinations.

many of the metabolic processes in several experiments (Jefferies, 1973; Marschner et al., 1981), chickpea has failed to accumulate Na^+ at the cost of K^+ in leaf and stem tissue, which also indicates its salt-sensitive nature (Rush and Epstein, 1981).

It is evident from the table that Ca^{2+} levels increase in all plant parts under K-deficient conditions, most prominently in the shoot region. According to Marshner (1986), when K-supply is abundant, 'luxury consumption' of K often occurs, and it possibly interferes with the uptake and physiological availability of Ca and Mg. It is quite possible that under conditions of K-deficiency an opposite pattern may appear. The increase in Ca^{2+} content at this stage may possibly contribute to maintaining the ionic balance of the cell.

It is well evident from Table 2 that P-total and Mg^{2+} are lowered in leaf tissue whereas they show increments in stem and root tissues due to K-deprivation. Similar findings have been reported by Repka (1983) in leaves and chloroplasts of maize plants under conditions of K-deficiency. Phosphorus is linked with energy transfer, whereas magnesium is mainly related with the activation of various enzyme systems (Epstein, 1972). From the way both these elements are excluded from the main metabolic organ (leaf) and accumulated in such other plant parts as stem and root tissue, it is quite clear that both are unable to overcome the situation of K-deficiency at the metabolic level.

Table 2 shows that iron and chloride levels are lowered in leaf and stem tissue, contrary to their accumulation in roots of K-deficient plants. The changes brought about by K-deficiency in manganese contents are very insignificant and do not indicate any disturbance in the Mn mediated reactions.

Organic constituents

The influence of K-deficiency on the carbohydrate status in chickpea plants is presented in Table 3. It is evident from the table that all carbohydrate fractions are increased in K-deficient leaf tissue. Non-reducing sugars and starch showed a decrease in stem tissues while reducing and non-reducing sugars are enhanced in root tissues due to omission of K. Starch content showed a decrease in K-deficient root tissue. The role of K in carbohydrate metabolism is very well documented and

Table 3

Effect of potassium deficiency on carbohydrate levels in chickpea (Cicer arietinum)

Plant Part	Treatment	Carbohydrates (g 100 g^{-1} dry tissue)				
		Reducing sugars	Non-Reducing sugars	Total sugars	Starch	Total
Leaf	Complete	0.914	3.270	4.184	13.816	18.000
	Potassium deficient	1.132	3.552	4.684	14.605	19.289
Stem	Complete	0.869	2.447	3.316	18.750	22.066
	Potassium deficient	0.869	2.078	2.947	17.961	20.908
Root	Complete	0.495	0.558	1.053	11.842	12.895
	Potassium deficient	1.184	1.711	2.895	10.263	13.158

Data represent average of three determinations.

Evans and Sorger (1966) reported that activities of nearly 19 different enzymes of carbohydrate metabolism are related to the presence of K. The accumulation of carbohydrates due to K-deficiency has been confirmed many times (Radi et al., 1973; Scherer et al., 1982) and this increase is mainly because of increased levels of reducing sugars, which play an active part in the osmoregulatory process. Present findings of increased reducing sugar levels especially in leaf tissues indicate that these might play a similar role. The increase in starch level in leaf tissue and its decrease in root tissue under K-deficiency may be due to differential effects of K-deficiency on amylase and starch synthetase activities. The accumulation of various carbohydrate fractions in K-deficient chickpea plants may also be due to reduced sink size (i.e. pod number and pod size).

It is well evident from the Table 4 that K-deficiency has differentially affected various nitrogen fractions. Total nitrogen, nitrate and free proline contents are lowered in K-deficient chickpea leaf tissues whereas in root tissues these compounds show enchancement. The soluble protein content is decreased in K-deficient leaves. Our results recall the work of Botrill and Possingham (1969) and Sinha and Singh

Table 4

Effect of potassium deficiency on nitrogen components in chickpea (Cicer arietinum)

Plant Part	Treatment	Total Nitrogen	Soluble Proteins	Nitrate	*Free Proline*
		(g 100 g^{-1} dry tissue)		(μg 100 g^{-1} dry tissue)	(mg 100 g^{-1})
Leaf	Complete	1.314	0.248	82	81.25
	Potassium deficient	1.012	0.210	68	60.31
Stem	Complete	0.767	—	52	60.63
	Potassium deficient	1.454	—	50	58.13
Root	Complete	0.581	—	40	45.06
	Potassium deficient	1.221	—	80	50.63

— Values not recorded.
Data represent average of three determinations.

(1984) who noticed a similar decline in total nitrogen in spinach and Japanese mint, respectively. The accumulation of total N in stem and root tissues and its decline in leaf tissues indicate that potassium deficiency probably exerts a negative influence on the transport of this element. The nitrate content shows a similar pattern, suggesting an arrested translocation process of this metabolite (Sinha and Singh, 1984). Moreover, it is apparent that these alterations in nitrate levels may further bring about marked changes in the nitrate reduction process in K-deficient plants. Low levels of N in leaf tissues due to K-deficiency might result from arrested protein synthesis (especially enzyme protein), as in the present investigation a decrease in soluble protein level is noticed in K-deficient chickpea leaf tissues. It is well known that under various stress conditions (heat, drought, salt) plants accumulate free proline which acts to balance osmoticum across the tonoplast. Goering and Thien (1979) have observed an increase in proline content in roots and shoots of maize seedlings subjected to deficiencies of nitrogen, phosphorus and potassium. Tang et al. (1985) have suggested a possible physiological role of proline towards the protection of plants from injurious effects of K-shortage. However, in the case of chickpea proline might possibly not play any major role under K-deficient conditions, because the plants do not show any tendency to accumulate this amino acid after being deprived of potassium. It is evident from the foregoing account that the status of inorganic as well as organic constituents in chickpea plants is altered markedly under the conditions K-deficiency, and this in turn can become responsible for various metabolic disorders.

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CELL MEMBRANE STABILITY AND ITS RELATION WITH SOME PHYSIOLOGICAL PROCESSES IN WHEAT

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A pot culture experiment was conducted with three levels of soil moisture contents (100–95, 60–55 and 30–25% of the field capacity) to measure cell membrane stability (CMS) in three wheat varieties by the polyethylene glycol test. The CMS of plants was measured after 45 days growth and compared with leaf water potential, osmotic potential, stomatal resistance (diffusive resistance), relative water content, excised leaf water loss and dry weight of above ground parts of plant. The concentrations of nutrient in leaf tissue, cell sap and cell leachate were determined. The CMS correlated well with leaf water potential, osmotic potential, water loss from excised leaf tissue and dry weight of plant. The CMS appeared to be influenced by osmotic potential of leaf tissue. Sugar and potassium were the major contributor to osmotic potential. The PEG test is a reliable method for measuring drought tolerance in wheat.

Keywords: cell membrane stability, water relations, stomatal resistance, nutrients

Introduction

Genetic improvements for drought resistance in crop plants depend on the identification of drought resistance mechanisms and the development of a suitable method for their measurement. Two major mechanisms of drought resistance are drought avoidance and drought tolerance. The former is manifested by a relatively smaller reduction in tissue water potential, and the latter is an ability of the plant tissue to sustain a smaller reduction in physiological and metabolic activity under decreasing water potential.

Bewely (1979) reported a critical role of cell membrane stability (CMS) under conditions of moisture stress as a major component of drought tolerance. The rate of injury to cell membranes may be estimated through the measurement of electrolyte leakage from the cells. A method for measuring cell membrane stability was found by Sullivan (1972), modified from that of Dexter's (1956) method. It is based on *in vitro* dessication of leaf tissue by a solution of PEG. After dessication, the electrolyte leakage into an aqueous medium is determined by the conductivity measurement. The degree of membrane stability to stress, which is evaluated by ion leakage, correlates well with tolerance of other plant processes to the stress in sorghum (Sullivan and Ross, 1972).

The genetic variability and heritability of CMS measured by this method have been studied in several crops. Martineau et al. (1979) concluded that a substantial variability existed in soybean and the selection of heat tolerant genotypes was feasible. Permachandra and Shimada (1987) reported a high heritability of CMS in orchard grass and found the technique efficient for selecting drought-tolerant-plants. The CMS of wheat was suggested to be controlled by polygenic action

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(Permachandra and Shimada, 1988). The genetic variability for drought and heat tolerance, as measured by this method, has also been reported in sorghum (Blum and Ebercon, 1979) and wheat (Blum and Ebercon, 1981).

The factors involved in resistance to dessication in PEG are mainly cell internal factors, such as osmotic potential of the leaf tissue, which resist osmotic stress of PEG solution. In addition, cell membrane quality, cell wall thickness and quality and cuticular thickness may influence response to dessication treatment. Natural dehydration can be measured as excised leaf water retention capability and water content (Dedio, 1975) which is mainly influenced by cuticular and stomatal resistance. A comparison of these and other physiological processes with CMS, measured by the PEG test, would help in understanding the physiological processes involved in differential ion leakage.

An experiment was conducted to compare the CMS measured by the PEG test, with some physiological measurement, and to examine a relationship of plant processes to ion leakage in wheat.

Materials and methods

Three wheat cultivars, DS-3, DS-4 and DS-17, were acquired from the genetic section of the Atomic Energy Agricultural Research Centre, Tando Jam. They were grown in an open pot house during Nov. to Jan. 1990 in plastic pots having a diameter of 22 cm with 35 cm depth. The pots were filled with 4 kg of alluvial soil with a field capacity of 22% (taken as 100) and a standard dose of NPK. The plants were thinned to three uniform seedlings per pot, 15 days after emergence. Pots were irrigated with deionised water on alternate days throughout the experiment.

The moisture content of the soil was maintained at three different levels, 100–95, 60–55 and 30–25, by weighing the pots daily on a balance, and the loss of water from the open surface of soil was calculated. The experiment was conducted in a randomised complete block design with three replicates. Measurements were taken when the plants were 50 days old, using the fully expanded second leaf from the top.

PEG test

Discs of 1.0 cm from a sampled leaf were taken using a leaf punch. Ten leaf discs were placed in a 30-ml test tube and washed three times with deionised water. For the dessication treatment, leaf discs were submerged in 30 ml of 40% solution of PEG-6000 and allowed to stand in solution for 24 h at 10 °C. The leaf discs were then washed three times with deionised water and both dessicated and non-dessicated control samples were allowed to stand overnight in 30 ml of deionised water at 10 °C. The tubes were warmed to 25 °C and shaken well, and the electrical conductivity was measured using an electric conductivity meter. After conductivity measurements, the leaf tissues were killed by autoclaving for 15 min and the electrical conductivity was measured at 25 °C. Three replicates were measured for the dessication treatment (T) and non-dessicated control (C). In addition, three replicates were made from the dessication treatment for the analysis of cell leachate.

The cell membrane stability of leaf tissues was calculated as the percentage injury using the equation, percent injury $= [1-(1-T_1/T_2)/(1-C_1/C_2)] \times 100$ where T_1 and T_2 = first and second conductivity measurement of dessication treatment, respectively and C_1, C_2 = first and second conductivity measurement of non-dessicated control, respectively.

Leaf water potential and osmotical potential

The leaf water potential was determined using a pressure chamber (OSK 2710, Japan). Measurements were done between 10.00 and 13.00 h. For the osmotic potential, leaf samples were separated from the plant and killed immediately in chloroform vapours, and the osmotic potential of extracted leaf sap was determined (Slavik, 1974) using a micro-osmometer.

Relative water contents (RWC)

Immediately after separation, the leaves were brought to the laboratory, weighed (F.wt) and allowed to stand overnight in a tube with half of its portion submerged in water. Each leaf was then removed the tube and, after water was gently absorbed from its surface with a tissue paper, it was weighed (T.wt) and dried in an oven at 70 °C for 24 h, then weighed (D.wt) again. The relative water content of the leaf was calculated by a relation (Weatherly, 1950).

$$RWC = \frac{F.wt - D.wt}{T.wt - D.wt} \times 100$$

Water loss of excised leaves

All leaves were sampled from one plant grown under different soil moisture content. These leaf tissues were weighed immediately, allowed to dessicate at 24 °C + 2 °C in a dark room, and reweighed, then oven-dried at 70 °C and weighed again.

Diffusive resistance

The leaf diffusive resistance was measured between 10.00 to 13.00 h using a Li-cor autoporometer (Li-cor Co. USA).

Nutrient analysis in dry matter, cell sap and cell leachate

Leaf dry matter, cell sap and cell leachate were analysed for sugar, Na, K, Ca, and P concentrations. Total sugars were analysed by the anthrone method (Yemm and Willis, 1954). Sodium, potassium and calcium were measured by using a flamephotometer. The total phosphorous was determined by the molybdenum venadate method.

Results

The injury percentage under PEG test in three wheat cultivars grown in three soil moisture levels is shown in Fig. 1(b). The varieties DS-4 showed the lowest and DS-17 the highest injury percentage in the PEG test, while DS-3 was non-significantly different from these varieties. The injury percentage decreased in plants grown under low soil moisture contents.

The injury percentage in the PEG test was significantly correlated with leaf water potential ($r = 0.899^{**}$) and osmotic potential ($r = 0.955^{**}$). Leaf water and osmotic potentials decrease relative to the lessening of soil moisture contents (Fig. 2, c and 1, d). Negative correlations were observed between injury percentage in the PEG test and stomatal resistance ($r = -0.868^{**}$, Fig. 1, b). The stomatal resistance increased under low soil moisture contents.

The relative water contents of leaf were negatively correlated, while the water loss from excised leaf tissues was significantly correlated with injury percentage in the PEG test ($r = 0.543^{*}$, $r = 0.849^{**}$, Fig. 2, a and 2, b). Relative water contents and water loss from the excised leaf decrease as soil moisture content lessens.

The dry weight of above-ground parts of plants was significantly correlated with injury percentage in the PEG test ($r = 0.8746^{**}$, Fig. 1, c). The dry weight of varieties decrease relative to the increase in soil moisture contents.

The concentration of sugars and different ions increased under low soil moisture contents and was negatively correlated (-0.894^{***} for Potassium and

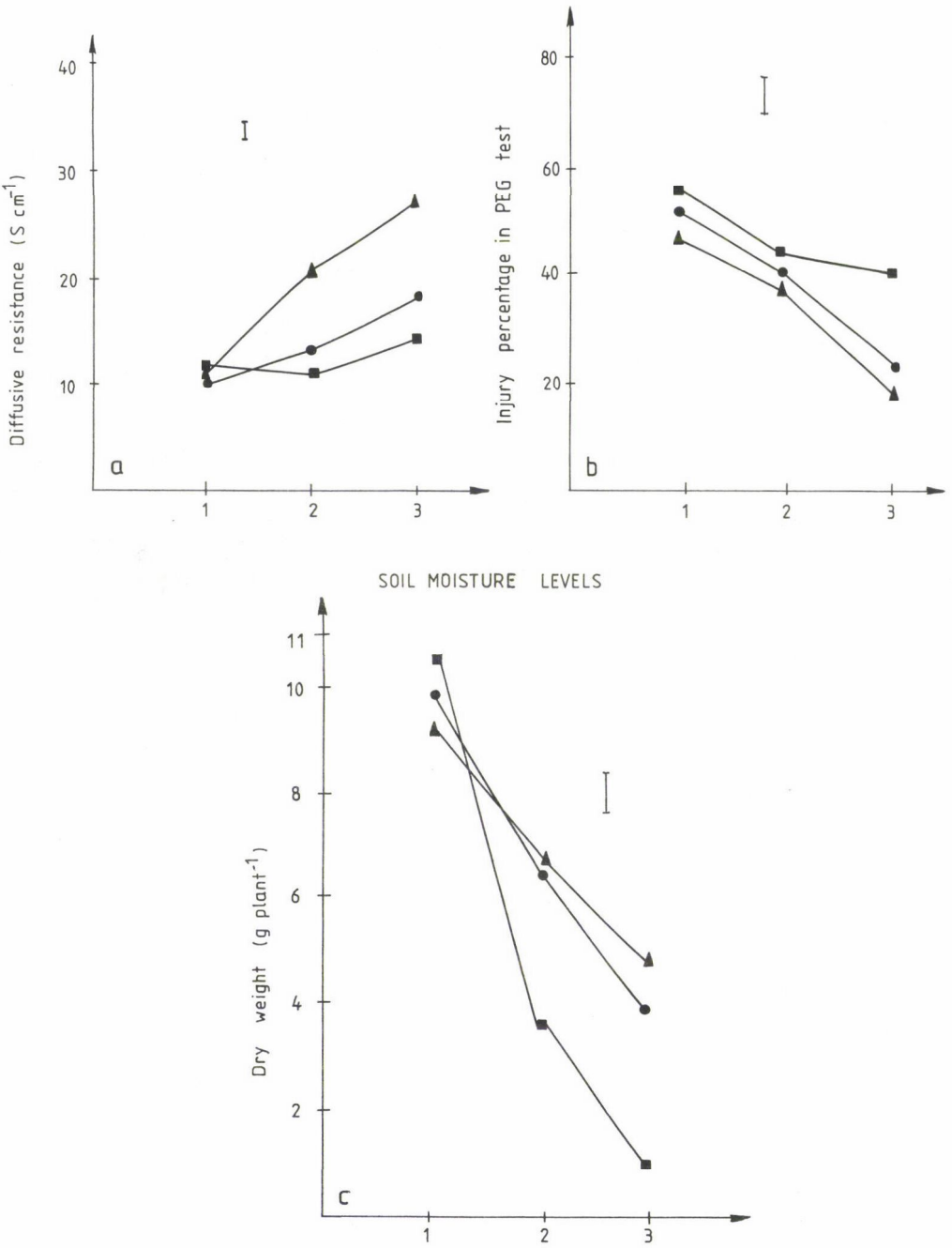

Fig. 1. Effect of different levels of moisture stress on (a) Diffusive resistance, (b) in jury percentage in the PEG test, (c) dry weight per plant in three wheat varieties; ▲ DS-4, ● DS-3 and ■ DS-17 grown under three soil moisture levels. (1) Field capacity, (2) 60 to 55%, (3) 30 to 25% of the field capacity. Vertical bars represent the LSD at 5%

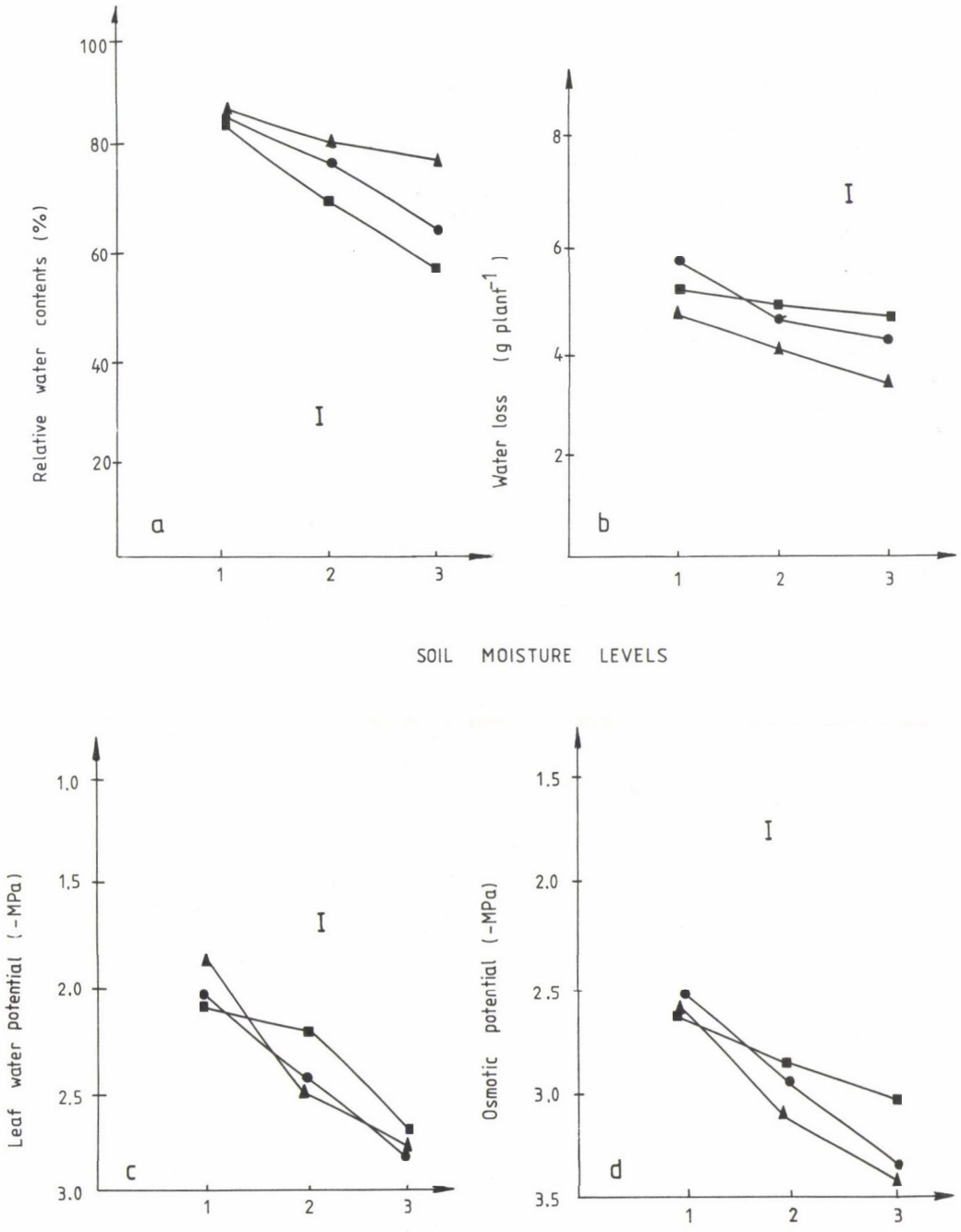

Fig. 2. Effect of different levels of moisture stress on (a) Relative water content (b) Water retaining capacity in the form of water loss (g plant^{-1}), (c) leaf water potential (d) osmotic potential in three wheat varieties; ▲ DS-4, ● DS-3 and ■ DS-17 grown under three soil moisture levels. (1) Field capacity, (2) 60 to 55%, (3) 30 to 25% of the field capacity. Vertical bars represent the LSD at 5%

Table 1

Relative solutes contribution to osmotic potential in three wheat varieties grown under three soil moisture levels

Soil moisture levels (%) of the field capacity and varieties	Na	K	Ca	P	Total sugars
	Concentration of the solutes in plant material m mol/gm dry wt				
DS-3					
100—95	1.67	63.97	6.33	1.70	84.40
60—55	2.11	74.37	7.60	2.73	137.90
30—25	2.22	87.03	8.76	3.77	179.13
DS-4					
100—95	1.87	72.10	7.11	2.44	101.03
60—55	2.13	94.40	8.87	4.36	178.37
30—25	2.68	114.30	11.65	4.92	212.43
DS-17					
100—95	1.35	62.20	6.78	2.07	88.83
60—55	1.24	70.47	8.17	2.31	109.37
30—25	2.04	83.13	8.97	2.59	153.70
LSD at 5%	0.2887	7.346	0.877	0.163	10.00

-0.939^{***} for sugar) with injury percentage in the PEG test for leaf tissue and for cell sap (Tables 1 and 2), respectively. The contribution of sugars, K, Ca, Na and P to osmotic potential increased under low soil moisture contents. Sugars and potassium were the main osmotic contributors, and sugars contributed more than did potassium to osmotic concentrations under low soil moisture contents.

The solutes of leachate generally declined with the increasing soil moisture content (Table 3) and the percentages of sugars were relatively higher than K, Ca, P and Na. Since the concentration of Na was very low (Table 1), K and Ca were likely the major solutes that affected the electrical conductivity measurement in the PEG test.

Discussion

The cell membrane stability test (CMS), for measuring drought tolerance by the PEG test, has been used successfully in sorghum, maize and wheat species (Blum and Ebercon, 1981). A comparative study made for measuring CMS in wheat constitutes three groups of varieties, i.e. DS-4 tolerant, DS-17 non-tolerant and DS-3 intermediate, since these differ non-significantly from the preceding (Fig. 2, a). The injury percentage in leaf tissues of plants grown under low soil moisture decreased, and this might have been due to acclimation of tissues to a lower water

Table 2

Relative solutes contribution to osmotic potential in three wheat varieties grown under three soil moisture levels

Soil moisture levels (%) of the field capacity and varieties	Na	K	Ca	P	Total sugars
	Concentration of the solutes in cell sap (m mol/l)				
DS-3					
100—95	0.127	221.94	15.04	16.74	207.17
60—55	0.130	192.81	12.98	13.86	192.97
30—25	0.127	232.80	19.40	20.41	210.67
DS-4					
100—95	0.123	252.50	19.65	21.90	227.81
60—65	0.137	234.63	15.09	21.73	213.11
30—25	0.140	257.81	22.15	29.54	246.58
DS-17					
100—95	0.103	168.18	11.78	11.09	180.45
60—55	0.103	163.30	11.69	11.18	188.22
30—25	0.110	170.37	11.83	11.14	186.21
LSD at 5%	0.0	3.375	0.661	1.477	4.529

Table 3

Percentage of solutes leached from leaves during the PEG test in three wheat varieties grown under three soil moisture levels

Soil moisture levels (%) of the field capacity and varieties	Na	K	Ca	P	Total sugars
	%				
DS-3					
100—90	85.38	50.08	81.60	6.82	28.31
60—65	78.96	41.18	75.23	4.86	18.55
30—25	75.04	37.21	62.57	3.85	16.98
DS-4					
100—95	81.66	37.09	61.58	5.97	20.72
60—55	61.66	31.71	55.83	4.45	16.27
30—25	54.09	32.16	49.59	4.05	12.71
DS-17					
100—95	87.35	55.62	85.36	7.75	30.73
60—55	84.30	49.58	80.89	6.58	24.89
30—25	87.00	39.96	74.39	5.55	19.40
LSD at 5%	1.2016	1.965	0.951	0.5689	0.9854

deficit (Permachandra et al., 1989). Membrane adjustments to drought have been reported in some lower plant forms (Bewly, 1979) and it may be that this phenomena also occur in wheat.

The injury percentage in the PEG test was significantly correlated with leaf water ($r = 0.988^{**}$) and leaf osmotic potentials ($r = 0.955^{**}$). The leaf water and osmotic potentials decreased under low soil moisture content, indicating a possible osmotic adjustment (Fig. 2, c and 2, d). The extent to which osmotic adjustments take place in three varieties differed. The primary effect of drought on the membrane is a loss of turgor pressure, changing the mechanical stress on the membrane and its interaction with the cell wall (Saxton et al., 1980). The stomatal resistance was negatively correlated to injury percentage in the PEG test ($r = 0.868^{**}$) and, in the order of cultivars, differences remain the same. Stomatal resistances (diffusive resistance) increase under low soil moisture contents (Fig. 1, b). The loss of water from excised leaf tissues decreases under low soil moisture contents (Fig. 2, b) and is highly significantly correlated to injury percentage in the PEG test ($r = 0.849^{***}$). The low loss of water from excised leaf tissues would indicate their ability to retain a higher water content, as is evident from Fig. 2, b. Since water loss from leaf tissues and their water contents are closely related characters (Deido, 1975), the water retention ability may be related to the protoplasmic permeability of a tissue, which varies among wheat cultivars (Oleinkova, 1970).

The dry weight of above-ground parts of plants decreases under low soil moisture contents (Fig. 1, c) and is highly significantly correlated with injury percentage in the PEG test.

The more favourable water status, which is manifested by an increase in stomatal resistance and higher water content, together with higher dry weight, indicates the same ranking of cultivars as in the PEG test.

Total sugars and potassium concentrations were negatively correlated with injury percentage in the PEG test, and increased under low soil moisture contents both in cell sap and leaf tissue (Tables 1 and 2). Potassium is an important ionic contributor to osmotic adjustment in water-stressed leaves (Turner, 1979; Jones et al., 1980). The contribution of sugar, potassium, and calcium increased under low soil moisture levels. Sugars and potassium were the primary contributor to osmotic adjustment, and sugars contributed more than potassium. While the primary osmoticum differs with crop, sugars and potassium are the primary contributors to osmotic adjustment in many plant species (Itoh and Kumura, 1987).

The leachate of solute declined with decreasing soil moisture contents (Table 3). Percentages of sodium, potassium and calcium were relatively higher than sugars and phosphorous. Since the concentration of sodium (Tables 1 and 2) was very low, potassium and calcium were the major ions that affected electrical conductivity measurement in the PEG test.

The results described above indicate that CMS measured by the PEG test was significantly correlated with leaf water potential, osmotic potential of leaf tissue, excised leaf water loss and dry weight of plant in different soil moisture contents.

Osmotic adjustment may have occurred in water-deficit plants, which might have affected solute leakage. Sugars and potassium were the major contributors to osmotic adjustment.

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EVALUATION OF RATES AND METHODS OF $^{65}ZnSO_4$ APPLICATION TO MUSTARD (*BRASSICA JUNCEA* L.) IN A SUB-TROPICAL CLIMATE

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The effects of rates and methods of zinc application on yield, and oil yield of mustard, and the percentage utilization of applied zinc by mustard, were studied in a field experiment on a zinc-deficient alluvial sandy loam soil (inceptisol) under subtropical climatic conditions using $^{65}ZnSO_4$. Zinc sulphate was applied as broadcast at 2.5, 5.0 and 10.0 kg Zn/ha; through irrigation water at 2.5, 5.0 kg Zn/ha and sprayed on the foliage as 0.1 and 0.2% solution corresponding to 1.0 and 2.0 kg Zn/ha, respectively. All the methods and rates of Zn application to mustard were found to be significantly superior to control (no Zn) treatment, in increasing the grain, straw and oil yield, as well as the Zn content of the plant. Foliar application of 0.1% zinc sulphate solution at flower initiation stage was found to be the best treatment in all respects. The soil solution concentration of Zn decreased with the progress of crop growth.

Keywords: mustard, Zn-deficiency, Zn-fertilizer, Zn-uptake

Introduction

Mustard (*Brassica juncea*), groundnut (*Arachis hypogea*), coconut (*Cocos mucifera*) and rapeseed (*Brassica nigra*) are the most common edible oilseeds of Southeast Asia. Edible oils are an integral part of the human diet as they provide an economical source of energy, particularly for the vegetarian population of the Southeast Asia. In recent years, efforts have been made to attain self-sufficiency in the production of edible oils in the developing countries. The oil content of mustard usually ranges between 40—50%, depending on the genotypes, environment and management practices. Response to irrigation is not very high in the case of old traditional varieties, while with the introduction of high yielding strains, the crop usually responds to irrigation (Prihar et al., 1981) depending upon soil type, winter rains and profile moisture.

The average yield of *Brassica* in subtropical regions of India, as compared to those obtained in Canada and Sweden, is very low. This poor yield of mustard in Southeast Asia is attributed to the fact that the crop is grown mostly in marginal lands of poor fertility, with a bare minimum input of water and fertilizers. The productivity and biosynthesis of oil depend largely upon prevailing environmental factors, including humidity and temperature, which greatly influence the duration of crop and consequently the oil yield (Gross and Stefanson, 1986; Canvin, 1965). Besides the depressive and additive effects of various environmental factors, a nutrient imbalance in plants was also found to affect the nitrogen metabolism, the protein and the oil synthesis by the crop (Dev et al., 1981). Mustard, being an oilseed

crop, responds well to fertilizer nutrients like N, P, B, S, Zn and Fe (Aulakh et al., 1980; Kumar and Gangwar, 1984; Holmes and Amery, 1978).

The deficiency of zinc is widespread in several crops and soils of the world (Randhawa and Nayyar, 1982; Takkar, 1982). Soil or foliar application of zinc sulphate is generally recommended to correct this deficiency. However, very little information is available on efficient rates and methods of zinc application for mustard in zinc-deficient soils. The objective of the present investigation was: a) to evaluate the effect of different rates and methods of zinc application on grain and oil yield of mustard; b) to determine the zinc fertilizer use efficiency by the crop under different rates and methods of the application; and c) to evaluate the effect of soil solution concentration of zinc at varying stages of crop growth on grain yield, Zn content in grain and fertilizer zinc utilisation by the crop in a zinc-deficient soil.

Materials and methods

The field experiment was conducted during 1987—88 on mustard (cv Pusa Bold) on an alluvial soil (inceptisol) in the Main Block (8C) at the Research Farm of the Indian Agricultural Research Institute, New Delhi (28.4° N, 77.1° E and 218 m altitude). The important soil characteristics were: pH 7.8, texture sandy loam, EC 0.45 S m^{-1}, organic carbon 0.33%, CEC 9.6 meq/100 g of soil and DTPA extractable Zn, Mn, Fe and Cu were 0.56, 3.6, 30.0 and 4.0 mg/kg soil, respectively. The soil properties were determined as per standard procedures, and available Zn, Mn, Fe and Cu were extracted with DTPA (Lindsay and Norvell, 1978) and estimated by AAS.

There were nine treatments, consisting of three different methods of application with different zinc levels and one control. The treatments were given as per the schedule in Table 1. Zinc fertilizer was applied as $ZnSO_4 \cdot 7\ H_2O$. The radioactive $ZnSO_4 \cdot 7\ H_2O$, prepared by tagging the fertilizer zinc with ^{65}Zn at the rate of 0.5 mCi/g Zn was applied in microplots of 1 m × 1 m size. Microplots were made at the centre of each main plot of 4.5 m × 4 m, by separating them from the rest of the main plot using rigid plastic sheets of 1 mm thickness, maintaining a height of about 20 cm above the ground and down to a depth of about 30 cm below the surface. In the case of soil application, the microplots received the $^{65}ZnSO_4$, whereas the rest of the main plot received non-radioactive zinc sulphate at the time of sowing.

The surface application of zinc fertilizer was done as a top dressing of zinc sulphate at the time of first irrigation, after 30 days of sowing. The spray application of zinc sulphate was given after two months of sowing, along with insecticide metacystox [Oxydemeton-methyl (S-(2-ethyl-sulphinyl)-ethyl) O-O, dimethyl phosphoro-thionate] at the flower initiation stage.

A basal dose of N, P_2O_5 and K_2O at the rate of 60, 40 and 40 kg/ha was applied at the time of sowing through urea, DAP and muriate of potash, respectively. The sulphur addition through zinc sulphate was balanced in other treatments through application of gypsum ($CaSO_4 \cdot 2H_2O$). The treatments were replicated four times in a randomised block design. The crop was sown with row-to-row spacing of 50 cm, and plant-to-plant 15 cm. In all five irrigations of approximately 60 mm, each were given at an interval of about one month. The Zn content of irrigation water was less than 5 ppb (μg/litre water). The crop received a total of 101 mm of winter rainfall during the growth period. The second dose of nitrogen at the rate of 40 kg/ha was topdressed at flowering stage.

The average minimum and maximum temperatures during crop growth period were 6.2° to 20.1 °C and 22.4° to 37.4 °C, respectively. The crop was harvested at maturity, and the total yields of grain and straw for each treatment were recorded. The soil and plant samples were collected after the harvest of the crop. Plant samples were washed successively with 0.01 N HCl, distilled water and deionised water. Both grain and plant samples (straw) were dried in a hot air oven at 60—65 °C. The straw samples were then ground in a stainless steel mill. The grain and straw samples were digested in a triacid mixture of $HNO_3 : HClO_4 : H_2SO_4$ (9 : 2 : 1), and the total Zn content in digested extracts was determined by AAS. The ^{65}Zn in the digested extract was determined by a single channel analyser, connected to a NaI(Tl) detector. The oil content in the seed was estimated by pulsed NMR spectrometer (Bruker Model Minispec 20, West Germany). Soil samples were extracted with DTPA for

Table 1

Rates and methods of zinc application

Methods		
Soil application (at the time of sowing)	Surface application (at first irrigation)	Foliar application (at flower initiation stage)
2.5 kg Zn/ha	2.5 kg Zn /ha	1.0 kg Zn/ha (0.1% Zn SO_4 solution)
5.0 kg Zn/ha	5.0 kg Zn/ha	2.0 kg Zn/ha (0.2% Zn SO_4 solution)
10.0 kg Zn/ha	—	2.0 kg Zn/ha (0.1% Zn SO_4 solution sprayed two times)

the estimation of available Zn and ^{65}Zn. The utilization of fertilizer zinc by the crop was computed by an isotopic dilution technique.

Soil solution samples were collected from all the treatments using soil solution samplers (Type 1900, Soil Moisture Equipment Company, California) at three stages, i.e. at the time of sowing after fertilizer application, at flowering and immediately after the harvesting. The zinc content in the soil solution was estimated by AAS.

Results and discussion

Grain and straw yield

The grain and straw yield of mustard under different rates and methods of zinc application is presented in Table 2. It is evident from the data that the grain and straw yield of mustard increased significantly due to the application of zinc fertilizer, irrespective of the rate or method of zinc application. The different methods and rates of zinc application did not differ significantly. Results of the present investigation clearly indicate that mustard also responds to zinc application significantly, in zinc-deficient soils under sub-tropical climatic conditions, like other crops such as rice, maize, groundnut and wheat, as reported earlier by several workers (Takkar and Randhawa, 1978; Singh et al., 1983 a, b; Grewal et al., 1984; Sarkar et al., 1980, 1983). It may be mentioned that foliar application of zinc sulphate at 1 kg Zn/ha through 0.1% zinc sulphate solution showed great promise in increasing grain and straw yield, as it was highly economical and convenient to use along with the routine insecticide (metacystox) spray. The spray application of 1 kg Zn/ha was almost comparable to 2.5 kg Zn/ha soil application in terms of grain and straw yield, which was the next best economical rate and method of zinc application to mustard.

Table 2

Effect of method and rate of Zn application on mustard yield, Zn content, % Zndff and % utilization of zinc

Method of application	Rate of application	Yield (Q/ha)		Zn content (mg/kg)		% Zndff		Total fertilizer uptake (g/ha)	% utilization	Oil yield (Q/ha)
		Grain	Straw	Grain	Straw	Grain	Straw			
A. *Soil application*										
	2.5	15.98	71.61	66.5	27.0	9.5	38.5	77.89	3.1	6.15
	5.0	14.59	71.27	77.5	35.0	17.0	44.9	128.36	2.6	5.68
	10.0	13.95	72.37	71.0	36.0	37.0	60.7	189.43	1.9	5.47
B. *Surface application*										
	2.5	13.70	59.63	59.8	28.2	14.0	53.0	97.37	3.9	5.20
	5.0	14.86	73.79	61.8	28.0	18.8	39.0	97.45	1.9	5.86
C. *Foliar application*										
	1.0	16.39	74.27	73.0	22.7	25.6	60.6	126.47	12.6	6.35
	2.0	15.66	69.66	76.5	40.5	26.1	36.5	129.35	6.5	6.05
	2.0*	14.71	64.41	82.0	41.0	21.9	24.9	163.14	8.2	5.06
D. *Control*	0	10.59	56.12	55.5	16.2	—	—	—	—	4.08
C.D. at 5%		2.78	12.59	11.3	8.7	3.9	16.7	31.68	1.4	1.08

* Applied in two equal splits.

Oil yield

Oil yield data (Table 2) are very much similar to crop yield data. The highest oil yield was obtained when only 0.1% of zinc sulphate solution was sprayed to the crop and followed by 2.5 kg Zn/ha soil application treatment. On the whole, the application of Zn, irrespective of the rate and method of application, significantly increased the oil yield of mustard over the control treatment. Samui et al. (1981) reported that application of Zn led to increased oil content in mustard *per se*, which may be due to activation of NADPH dependent dehydrogenase involved in fat synthesis by Zn. Iweibo and Weiner (1972) also reported a similar involvement of Zn in the fat synthesis in oilseeds.

Zinc content

Regardless of the method and the rate of application, Zn significantly increased the Zn content of both grain and straw. The Zn content of mustard grain in the check plot was 55.5 mg/kg, and that of straw was 16.2 mg/kg, but it rose significantly to 82.0 and 41.0 mg/kg in grain and straw, respectively, through 2 kg Zn/ha foliar spray split application (Table 2). Sharma and Grewal (1988) also reported that Zn applied through soil or foliar sprays significantly increased the Zn content and Zn uptake of potato tubers over control.

Percent zinc derived from fertilizer (%Zndff) and fertilizer Zn uptake

The % Zndff increased with the increasing rate of zinc application, and the highest % Zndff in grain and straw was obtained at 10 kg Zn/ha through soil application (Table 2). The foliar application of zinc gave significantly higher Zndff in grain than did the surface application. The % Zndff in grain and straw at 0.1% $ZnSO_4$ spray application was highest amongst the three spray treatments, and next to 10 kg Zn/ha soil treatment (Table 2). The fertilizer Zn crop uptake showed the same trend as that of % Zndff in grain.

Percentage utilization of applied Zn

The utilization of applied Zn (Table 2) was maximum (12.6%) when Zn was applied through foliar spray at the rate of 1 kg Zn/ha (0.1 % $ZnSO_4$ solution), and it reduced to half (6.5%) when a double dose of Zn (2 kg Zn/ha) through 0.2% $ZnSO_4$ solution was applied. The utilization of fertilizer zinc was found to increase again from 6.5 to 8.2% when the applied dose (2 kg Zn/ha) was given in two splits. When the Zn was mixed with the soil (at the time of sowing), or applied to the surface (at the time of irrigation), the percentage utilization was quite low (1.9 to 3.9%) as compared to that of foliar application. It has been reported by several workers (Dwivedi et al., 1990; Singh and Kamath, 1989; Sreenivasa Raju and Kamath, 1982; Sarkar, 1979) that relatively immobile nutrients like P and Zn, when applied through foliar spray to the cereals, are not utilised efficiently by the crops, as was evident by the low dry matter yield of crops under such treatments. The present findings, however, indicate that the highest utilization of fertilizer zinc can be obtained in mustard through foliar application of zinc, which was responsible for giving a higher grain yield in the foliar spray treatment than control.

The results thus clearly reveal that the foliar application of zinc at the rate of 1 kg Zn/ha through 0.1% zinc sulphate solution at the flower initiation stage can meet the zinc requirement of mustard in a zinc-deficient soil.

Soil zinc

After the crop harvest, DTPA extractable zinc in soil increased to 3.51 mg/kg (Table 3) where a soil application of 2.5 kg Zn/ha was carried out. Other parameters showed almost the similar trend as that of plant Zn. The % Zndff was found to be maximum in soil samples which received 10 kg Zn/ha through soil application, and the recovery of applied zinc was found to be highest in soil samples where Zn was applied as 2 kg/ha through a foliar spray of 0.2% zinc sulphate solution, in two equal splits.

Table 3

Zn content, % Zndff, Fertilizer Zn content and % recovery of fertilizer zinc in DTPA extract of soil taken after the harvest of mustard crop

Method of application	Rate of application (kg/ha)	Zn content (mg/kg)	% Zndff	Fertilizer Zn (mg/kg)	Recovery (%)
A. *Soil application*					
	2.5	3.51	11.4	0.8	30.7
	5.0	3.22	20.0	0.62	24.9
	10.0	2.12	32.0	0.65	13.0
B. *Surface application*					
	2.5	2.49	17.3	0.41	33.0
	5.0	2.52	24.6	0.60	24.1
C. *Foliar application*					
	1.0	2.38	12.1	0.29	57.0
	2.0	2.37	15.0	0.34	34.4
	2.0*	2.47	17.0	0.78	78.3
D. *Control*	0	1.92	—	—	—
C.D. at 5%		0.67	7.8	0.093	9.6

* Applied in two equal splits.

Table 4

Zn content (μg/1) in soil solution samples collected at different growing stages of the crop

Method of application	Rate of application (kg/ha)	1st sampling (at sowing time)	2nd sampling (at flowering stage)	3rd sampling (after harvest)
A. *Soil application*				
	2.5	61.0	47.5	40.0
	5.0	55.0	50.0	36.3
	10.0	57.5	40.0	42.5
B. *Surface application*				
	2.5	61.0	52.5	28.8
	5.0	60.0	48.8	42.5
C. *Foliar application*				
	1.0	69.0	47.5	48.8
	2.0	67.5	58.8	41.3
	2.0*	55.0	58.8	37.5
D. *Control*	0	52.5	45	23.8

* Applied in two equal splits.

Zinc content in soil solution during growing stage

The concentration of zinc in soil solution determines the diffusive flux of zinc to the plant root as well as the magnitude of mass flow supply of zinc to the crop. The zinc content of soil solution samples taken at three different growth stages of the mustard crop are reported in Table 4. The zinc content of soil solution at the time of sowing under various zinc treatments did not show any significant variation, although the soil solution collected from control (no zinc) plots showed the lowest zinc content. There was a gradual decrease of zinc concentration in soil solution from the sowing to the harvest stage of crop growth. The Zn content of soil solution samples taken at the time of sowing showed significant positive correlation with grain yield and percentage utilization of applied Zn.

Such results are in accordance with the earlier findings of Sharma and Deb (1987), who reported increased utilization of fertilizer zinc for wheat crop with the increase of soil solution concentration of zinc. At the flowering stage, the Zn content of soil solution samples showed significant positive correlation with grain Zn content, and the recovery of applied Zn in the DTPA extract of soil samples collected after the harvest of the crop. No significant correlation was found between the Zn content of soil solution collected at the time of harvest and the plant and soil parameters studied. This is normally expected, as the dry matter yield and utilization of applied fertilizer by the crop are mainly controlled by the concentration of nutrients present in soil solution prior to the flowering and grain formation stages.

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PHENO- AND GENOTYPIC FLORAL CHARACTERS IN SEVERAL TAXA OF SOME GENERA IN THE SUBFAMILY *PRUNOIDEAE*

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The author has examined 152 stone fruit cultivars and varieties for 9 characters of flower morphology since 1968. The first thing he wanted to know was: which of them could be regarded as genotypic characters. The seasonal sensitivity of the stone fruits belonging to 32 taxonomic units varied with the size and position of flowers and even with the length of the peduncle. The seasonal changes are well represented by the frequency of apistillate flowers, so the ecological sensitivity—and genetic stability—in the flowers can be established on the basis of the standard deviation.

According to the investigations made so far, the correlations between the reproductive organs, the median of petal, the stamen number, the relative stamen number, the length of the external filaments and the frequency of abnormal flowers are suitable (though to a lesser extent) for the flower morphological description of varieties, the analysis of the progeny and the acceleration of the work of selection.

Keywords: floral compartments, pheno- and genotypic remarks, taxa of *Prunoideae*

Introduction

The detailed variety descriptions (Hedrick et al., 1911, 1915, 1917; Dahl, 1935; Röder, 1940; Tóth, 1957) and the taxonomic works of varieties (Hedrick, 1925; Brózik, 1959, 1960, 1962; Brózik and Regius, 1957) attach the same importance to the characteristics of the flower as do the practical pomologies (Dermine and Liard, 1957, 1978; Tomcsányi, 1979; Brózik, 1974). Some of the publications concerned with the genetic study of fruit-bearing plants also analyse the very characteristics of the vegetative and reproductive organs that may assist better descriptions of the varieties.

Thus, the breeding and pomological works of taxonomic view are of particularly great importance, since the environmental dependence of the different characters determines the success, time requirement and pomological usefulness of the purposeful breeding work. The seasonal effects appear differently in the characters; it was just by the least phenotypic features of flower morphology that we determined the morphological characters of self-fertility (Surányi, 1974, 1980a, 1989).

Our observations have mostly covered cultivated and historical plum varieties (Surányi, 1978), though we have also made numerous examinations among varieties of other species (cf. Surányi, 1980a, 1990a). In the case of selected plants of the progeny of some varieties, the pheno- and genotypic floral characters are able to accelerate the breeder's selection; the size of petal, the stamen number, the relative stamen number and the length of the external filaments, in particular, proved stable

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characters (Surányi, 1990b). Thus, the more than twenty-year studies of the genotypic floral characters have dealt with the plum; in the present study we wish to clarify whether our statements hold true of many taxa of the subfamily *Prunoideae* as well (cf. Kárpáti, 1967).

Materials and methods

The varieties and individuals chosen were collected in different periods between 1968 and 1990, according to an earlier described method and statistical processing system (Surányi, 1989). Nine characters of flower morphology were found to be important for characterizing the botanical and fertility conditions of the varieties. The taxa were characterized by values obtained from 4—5-year averages of individuals of varieties, subvarieties and forms of various number, and the year's effect was used to establish the standard deviation.

This value as well as the apistilly, the relative stamen number, are equally found in the summarizing table; the standard deviation was statistically analysed.

Regarding the different characters, in the flowers (30 per treatment) collected from bearing twigs of the individuals, varieties and forms chosen, we recorded the pistil length (mm), stamen number and relative stamen number (n/mm). In every 5th flower we determined the median of petal (mean value of length and width), the length of external filaments (mm) and the stigma diameter (μm/40x magnified). Furthermore, in samples taken from every 10th flower, we determined the size of pollen (160x) and, in 15% saccharose, the tube developing capacity (80x). Within the taxa chosen, the flower morphology characters of the different stone fruit varieties and trees were also evaluated by analysis of variance. Since we only wished to prove the usefulness of the characters without giving details—pointing out their genetic and year- and environment dependent (phenotypic) sensitivity—we used several comprehensive illustrations in the paper.

The taxa were characterized on the basis of the following varieties, individuals and years:

Amygdalus communis

convar. *communis* (sweet almond) (1976—1980): C. 431, C. 446, C. 447 and C. 471 rootstock variety

provar. *amara* (bitter almond) (1976—1980): C. 449 and C. 472 rootstock variety

Amygdalus nana (dwarf almond): Ceglédi ('Pörös') (1987—1990) and Csongrádi ('Temető') (1981—1985)

× *Amygdalopersica hybrida* (peach-almond) (1976—1980): C. 410 and C. 465 rootstock variety

Persica vulgaris (peach): Győztes, Homokgyöngye, Ford korai, Amsden, Cegléd szépe, Champion, Alexander, Elberta, J. H. Hale, June Elberta, Mayflower, Salwey, Shipley (1968—1971); C. 932 and C. 2629 rootstock variety (1977—1981)

Armeniaca vulgaris

convar *minor* (wild apricot) (1980—1984): C. 1426, C. 1301, C. 1652, C. 1870, C. 1620, C. 615, C. 195, C. 2546, C. 694, C. 1650, C. 809, C. 1300 rootstock variety

convar. *vulgaris* (apricot) (1980—1984): Gönci and Paksi Magyar kajszi, further, C. 235, C. 256, C. 1646 and C. 1789 Magyar kajszi

convar. *dulcis* (sweet apricot): C. 712 Mandula kajszi (1980—1984)

convar. *persicoides* (Rose apricot): C. 333 Borsi rózsa, C. 777 Hetényi rózsa, C. 778 Kecskeméti rózsa, C. 1478 Kései rózsa (1968—1971); C. 320, C. 326 Rózsakajszi, C. 671 Kécskei rózsa (1980—1984)

Armeniaca vulgaris f. *pendula* (1971—1974)

Armeniaca mume (mume apricot): Isfarak (1968—1970)

Armeniaca ansu (ansu apricot): H VII. and H. VIII. (1970—1972)

Armeniaca dasycarpa: C. 308 myrobalan apricot (1970—1972) and Plumcot (plum apricot) (1979—1981)

Prunus spinosa (blackthorn) (1983—1986): Dolinai (Albertirsa) I—IV.

Prunus insititia (bullace) (1978—1982): C. 83, C. 806 and C. 1422 bullace, C. 1253 Potyó szilva rootstock variety

Prunus cerasifera (myrobalan) (1980—1984): C. 162/a, C. 174, C. 359, C. 679, C. 801, C. 364 and C. 767 rootstock variety

Prunus domestica (plum) (1975—1980): Ageni, Besztercei szilva, Löweni szép, Tragédia, Tarka perdrigon, Bosznia királynője

Prunus italica
convar. *pomariorum* (round plum): Katalán szilva (1975—1980)
convar. *claudiana* (gage) (1975—1980): Bavay, Bolvilleri, Althann, Sermina, Uhinksz ringlója, Zöld ringló
convar. *ovoidea* (egg plum) (1975—1980): Nagy cukor, Montfort, Sárga tojás
convar. *mamillaris* (egg plum) (1975—1980): Kék datolya, Gömöri nyakas, Beregi datolya, Fehér királynő
Prunus syriaca convar. *cerea* (true mirabelle) (1975—1980): Korai kedvenc, Nancy mirabella, Metzi mirabella
Prunus salicina (Japanese plum) (1987—1989): Methley, Santa rosa, Duarte
Prunus nigra (Canadian plum) (1983—1986)
Prunus americana (American plum): De Soto (1987—1989)
Cerasus fruticosa (bushy cherry) Nagykőrösi (1984—1986)
Cerasus avium
ssp. *avium* (wild cherry) (1979—1983): C. 2493 and Altenweddingeni rootstock variety
convar. *juliana* (early cherry) (1968—1971): Münchebergi korai, Szomolyai, Májusi korai, Márki korai
convar. *duracina* (heart-cherry) (1968—1971): Hedelfingeni, Germersdorfi óriás, Badacsonyi, Jaboulay
C. avium f. *pendula* (1974—1977)
Cerasus vulgaris
convar. *acida* (black sour cherry) (1968—1971): C. 215 and C. 404 Cigánymeggy
provar. *vulgaris* (sour cherry) (1968—1971): C. 116 Pándy meggy, Nagy angol, Ostheimi, Újfehértói fürtös (1976—1980)
provar. *austeza* (sweet sour cherry) (1968—1971): Eugénia császárnő, Hortenzia királynő
Cerasus vulgaris f. *plena* (1972—1975)
Cerasus serrulata (Japanese cherry) (1972—1975)
Cerasus triloba cv Plena (1972—1975)
Cerasus mahaleb (mahaleb) (1976—1980): C. 500, C. 2753, Érdi V. and SL 64
Cerasus tomentosa (downy cherry) (1977—1979)
Cerasus besseyi (western cherry) (1976—1978)
Padus avium (may tree) (1976—1980): Ceglédi (GYDKFV) and Csemö-Putri-sarki
Padus serotina (late cherry) (1977—1989): Albertirsai and Mikebudai
Laurocerasus vulgaris (bay-cherry) (1976—1980): Ceglédi I (large-flowered) and II (small-flowered).

Results and discussion

Of the fertility and flower structure of many varieties of major species in some genera of the subfamily *Prunoideae*, little information is available. Furthermore, recently new aspects have even arisen with various species (cf. Orosz-Kovács, 1991; Surányi and Orosz-Kovács, 1992; Faust, 1989). The stability in size of the floral characters is rather diversified, e.g. in the genera *Amygdalus* and *Persica* a higher-than-15% fluctuation can be observed in the case of pistil length and apistilloidy, and the diameter of stigma and the rate of pollen tube formation also show a nearly 10% variation. Two varieties of the × *Amygdalopersica hybrida* form conspicuously unstable flowers. In the case of the C. 410 clone variety, the wide fluctuation of the fruit- and seed size primarily indicates the overdominance of the phenotypic features. The frequency of defective flowers is also the highest in these two genera (Table 1).

According to the evidence of the flower morphology characters studied, in the genus *Armeniaca* the stigma diameter and the pollen size, while in the *Prunus* species the pollen size and the pollen tube formation, were the most responsive to

Table 1

Important remarks of taxons in subfamily of Prunoideae

Taxon	Apistilly %	Effects of years %	Relative stamen number pc/mm	Significance
Amygdalus communis				
convar. *communis*	8—12	13.7	1.80	6, 8, 9
provar. *amara*	6—8	9.2	1.80	2, 6, 8, 9
Amygdalus nana	7—12	12.2	1.90	2, 6, 7
Amygdalopersica hybrida	7—12	21.0	1.40	2, 6, 8, 9
Persica vulgaris	6—9	9.2	1.90	2, 3, 9
Armeniaca vulgaris				
convar. *minor*	6—8	10.5	1.90	2, 6, 8
convar. *vulgaris*	3—4	8.2	1.70	6, 7, 8, 9
convar. *dulcis*	4—5	22.0	1.50	2, 6
convar. *persicoides*	6—8	14.4	1.60	2, 6
Armeniaca vulgaris				
f. *pendula*	7—15	12.8	1.80	2, 4, 6, 9
Armeniaca mume	2—3	10.7	2.10	2
Armeniaca ansu	2—3	9.7	1.95	3, 6
Armeniaca dasycarpa	7—10	17.8	2.90	2, 4, 6, 7, 8
Prunus spinosa	1—2	12.0	3.10	8
Prunus insititia	1—2	11.5	2.60	2, 4, 7, 8
Prunus cerasifera	2—4	18.8	3.80	2, 3, 4, 6, 7, 8
Prunus domestica	2—5	14.7	2.30	2, 6, 7, 8
Prunus italica				
convar. *pomariorum*	4—6	10.0	2.00	7, 8
convar. *claudiana*	6—8	14.8	2.40	6, 7, 8, 9
convar. *ovoidea*	2—4	13.2	2.20	7, 8
convar. *mamillaris*	7—10	14.3	1.80	4, 7, 8
Prunus syriaca				
convar. *cerea*	4—6	12.3	2.00	6, 8
Prunus salicina	4—8	9.0	2.20	2, 8
Prunus nigra	1—3	7.7	2.70	7, 9
Prunus americana	1—2	15.0	1.90	3, 7, 9
Cerasus fructicosa	3—5	11.1	2.10	3, 7
Cerasus avium				
ssp. *avium*	1—2	7.5	2.70	
convar. *juliana*	1—2	8.9	2.30	
convar. *duracina*	2—4	10.3	2.60	
Cerasus avium				
f. *pendula*	6—10	14.2	2.60	2, 4, 6, 7, 8
Cerasus vulgaris				
convar. *acida*	4—5	8.1	2.80	2, 8
provar. *vulgaris*	5—6	7.6	3.00	7, 8
provar. *austeza*	6—8	18.3	2.70	2, 7, 8
Cerasus vulgaris f. plena	6—8	12.0	2.90	4, 6, 7, 8
Cerasus serrulata	3—5	8.9	3.20	8
Cerasus triloba	6—10	7.1	3.40	9
Cerasus mahaleb	1—2	8.8	3.00	4, 7
Cerasus tomentosa	5—6	8.5	3.10	
Cerasus besseyi	5—8	9.3	2.70	7, 9
Padus avium	4—6	9.9	6.00	6, 7
Padus serotina	4—7	8.2	5.00	8
Laurocerasus vulgaris	1—2	8.3	3.80	6, 8

Note: 1 — Median of petal; 2 — Pistil length; 3 — Stamen number; 4 — Relative stamen number; 5 — Length of external filament; 6 — Diameter of stigma; 7 — Size of pollen; 8 — Pollen germination; 9 — Apistilly

the climatic (year's) effects. The sweet-stoned apricots (convar. *dulcis*), the *Armeniaca dasycarpa* and the *Prunus cerasifera* varieties showed wide yearly fluctuations. With the exception of several taxa, apistilloidy is very rare in these two genera, though in the more sensitive taxa this also sometimes exceeded the 10% frequency.

The genus *Cerasus* also showed some specific features; the relative stamen number is generally high in the taxa, secondary polyandria occurs very frequently (Orosz-Kovács, 1991), and the effects of years are practically insignificant. exactly as in the genera *Laurocerasus* and *Padus*. This is true even if some authors pointed out considerable year effects in certain sour cherry varieties (Pozvai, 1984), although the age of the tree and the topology of the flowers in the branch system may greatly influence them. However, these effects, compared with the role of certain pathological processes, are not significant (cf. Nyéki, 1980).

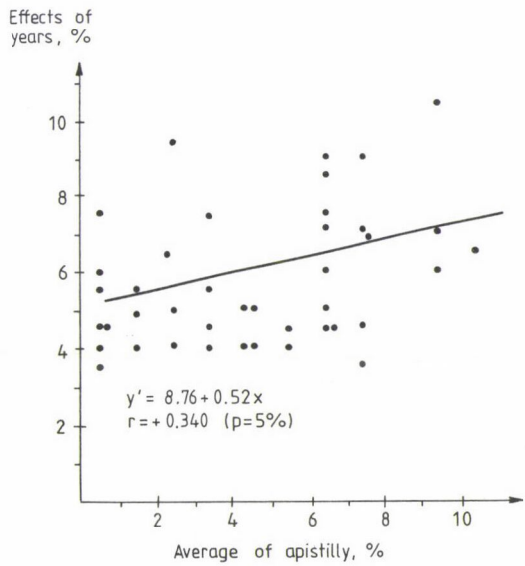


Fig. 1. The apistilly as an indicator of phenotypical remark in flowers of *Prunoideae* according to regression analysis (1968—1990)

The correlation between the yearly fluctuation of the floral characters and the tendency to apistilloidy in the 42 taxa is not merely apparent, although apistilloidy was only one of the 9 characteristic features. The correlation is rather close ($r = +0.340$, $p = 5\%$), and consequently, it is significant (Fig. 1). Similarly close is the correlation between pistil length and stamen number, according to the taxa grouped by five genera (Fig. 2, Table 2). They practically confirm our earlier observations that the correlation between gynoecium and androecium can be numerically proved (cf. Surányi, 1974, 1980a), although considerable differences by the quality of the correlation—depending on how the stamen number changed with a change in the pistil length—occurred. The genera, on the other hand, suggest an

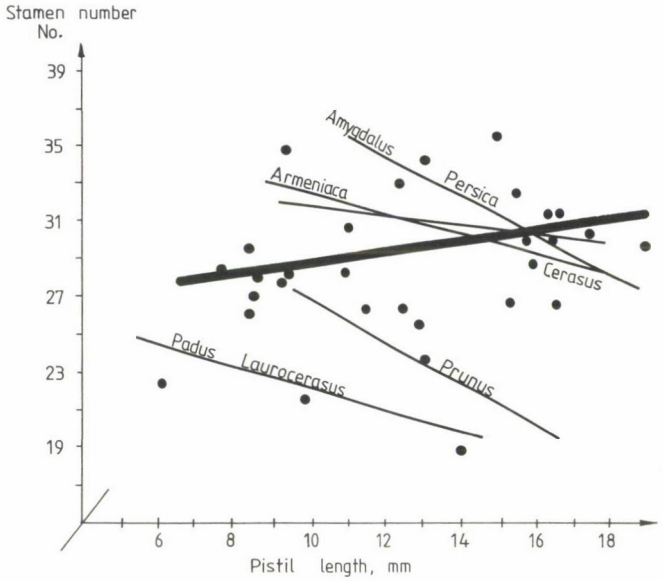
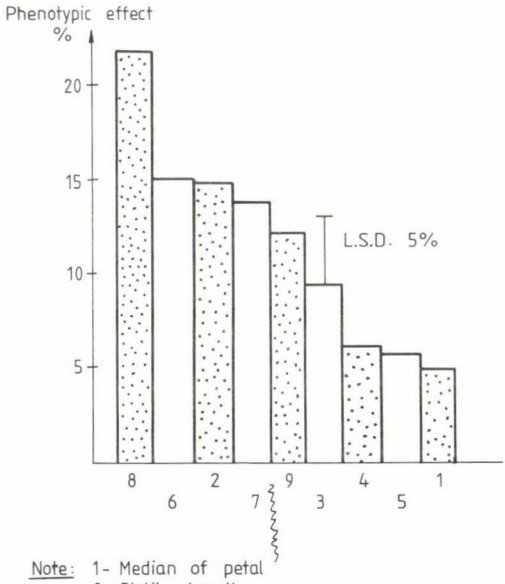


Fig. 2. Correlative effects between sexual organs



Note:
1- Median of petal
2- Pistil length
3- Stamen number
4- Relative stamen number
5- Length of external filament
6- Diameter of stigma
7- Size of pollen
8- Pollen germination
9- Apistilly

Fig. 3. Effects of years according to some flower remarks in taxons of *Prunoideae*

opposite trend; they cover a range of species from those with short-stemmed flowers set by 1 to 3, to species with relatively long-stemmed flowers forming the inflorescence ($r = +0.346$).

We established the extent of phenotypic effects by taking into consideration the fluctuation of 9 floral characters over 3 to 5 years in 152 cultivars of 42 species, subspecies as well as pro- and convarietas. Pollen germination was the character least controlled genetically; the diameter of stigma, the length of pistil and the size of pollen significantly differed both from the pollen germination and from the other

Table 2

Correlative effect in relationship of gynoecium (as pistil length) and androecium (as no. of stamens) in some genus Prunoideae

Genus	n	r-value
Amygdalus and *Persica*	110	−0.233*
Armeniaca	145	−0.505***
Prunus	208	−0.701***
Cerasus	121	−0.188^{+}
Padus and *Laurocerasus*	28	−0.998***
All together	612	−0.346***

$^{+}$ p = 10%
* p = 5%
*** p = 0.1%

characters not listed here. To summarize, the stamen number (cf. Morrison, 1964), the relative stamen number (Surányi, 1976), the length of filaments differentiating in the outer circle and the median of petal (Surányi, 1980b) are in essentials genotypic characters of the flowers of *Prunoideae* taxa (Fig. 3).

Thus, the morphogenetic characters can be used to characterize self-fertility in almond (Vasilakakis and Porlingis, 1984), plum varieties (Surányi, 1978), cherry (Surányi, 1990c) or even in sour cherries (Lansari and Iezzoni, 1990) and they can also be used in the selection of hybrids. The extension of investigations to fruits of other families and even to other cultivated plants may obviously represent an interesting line in future researches (cf. Schwanitz, 1973). Nor are the genotypic flower morphology characters without importance in regard to further investigations.

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THE EFFECT OF SPRAYING AND POST-HARVEST TREATMENT WITH CALCIUM CHLORIDE ON THE FRUIT QUALITY CHANGES OF "ANNA" APPLES DURING COLD STORAGE AT 4 °C

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Anna apple fruits were dipped in 3% $CaCl_2$ with and without vacuum (350 mbar); other fruits were taken from trees sprayed 3 times with 0.5% $CaCl_2$. All fruits stored for 50 days at 4 °C. The results indicates that fruits which previously dipped in 3% $CaCl_2$ under vacuum had a lowest respiration rate and were firmer than other treatments and control, followed by those dipped in 3% $CaCl_2$ without vacuum. Spraying treatment had no effect on the above-mentioned fruit characters.

Keywords: cold storage, $CaCl_2$ treatments

Introduction

Extensive work has been done on calcium and its effect on controlling physiological disorders (Poovaiah, 1986) and delaying senescence in fruits (Batts and Bramlage, 1977; Blanpied, 1981; Lee and Dewey, 1981; Poovaiah, 1986). It has been reported (Martin et al., 1975) that calcium is an important determinant factor of fruit quality. Vacuum infiltration of a $CaCl_2$ solution doubled the calcium content of fruits compared with a dip treatment in the same solution (Conway and Sams, 1983), and improved maintaining fruit firmness during cold storage and the vacuum infiltration was more effective (Poovaiah, 1986). Early season sprays (3.6 g/L $CaCl_2$) does not raise fruit calcium content as much as late season calcium sprays, but there were more effective in reducing fruit pitting of "Golden Delicious" apples (Stahly, 1986). Raese (1988) has reported that calcium spray materials substantially improved fruit firmness. The use of calcium dipping increased flesh firmness of "Jonathan" apples by 0.45 kg (Bangerth et al., 1972), but increased fruit firmness and reduced the rate of respiration in "Anna" apples (Attia, 1986) and in "Red Delicious" apples (Chopra and Naqash, 1986).

Golden Delicious apples pressure infiltrated with $CaCl_2$ were firmer than untreated fruits (Drake and Spayd, 1983; Laufmann et al., 1988) and no effect on respiration was observed (Carl and William, 1984). Post climacteric respiration of "Baldwin" apples decreased as peel calcium level increased from 400 to 1300 ppm (Bramlage et al., 1974). An inverse relationship between calcium content and respiration in apples (Faust and Shear, 1972; Faust, 1974).

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Materials and methods

This work was carried out in both 1988 and 1989 seasons using "Anna" apples budded on Balady rootstock grown et El-Shein, Kafr El-Sheikh Governorate, to study the effect of pre- or post-harvest application of $CaCl_2$ on the fruit quality of "Anna" apples.

The pre-harvest treatments were done by using 0 (as control) and 0.5% of $CaCl_2$. Trees were sprayed 3 times, every 3 weeks, beginning about 20 days after petal fall. Both treatments (0 and 0.5% $CaCl_2$) were replicated 3 times, each replicate involving 3 trees. The other two treatments were post-harvest treatments. The first post-harvest treatment was calcium vacuum infiltration of apples and was done as follows: apples were submerged in 3% (Wt./Vol.) solution of $CaCl_2$, the pressure was reduced to 350 mbar for 3 min then slowly released. In the second post-harvest treatment apple fruits were dipped for 3 min in 3% (Wt./Vol.) $CaCl_2$ solution, and the apples were dried at room temperature. In all treatments in both years mature apple fruits were harvested after 110 days following petal fall (27.6, 1988 in the first season and 24.6, 1989 in the second season). Fruit acidity percentage was about 0.8, TSS percentage was about 10.5% and fruit firmness was about 12 Lb./inch^2 (ADS, 1982). Then, treated as mentioned above and packed in 3 kg carton boxes, each treatment was represented with 5 cartons. The fruits were stored for 50 days at 4 °C, and 90—92% relative humidity commercial storage at Kafr El-Sheikh. One carton of each treatment was taken out of storage after fifteen and thirty days, then every 10 days. The following physical and chemical analysis were determined.

1—Flesh firmness: was measured with the skin removed, Magness—Taylor type pressure tester with 5/16 inch standard plunger, date in Lb/square inch recorded.

2—Respiration: 1 kg fruits placed in a discator and connected to a tube contains 25 ml of 1.0 N KOH air free from CO_2 was immersed into the discator through the KOH for one hour. Then KOH titrated with 1.0 N HCl using thymolblue indicator, CO_2 production calculated as mg CO_2/kg/h. The results were statistically analyzed according to Little and Hills (1972).

Results and discussion

Fruit flesh firmness

Fruit flesh firmness decreased in both storage seasons 1988 and 1989 through the storage period (Table 1). At the harvest date in both seasons, and during the storage period in the first season, no significant differences between foliar $CaCl_2$ sprays (retains 66.29% of its firmness at the harvesting date) and the control (retains 65.32% of its firmness at the harvesting date) on maintaining fruit firmness was observed; but at the end of the second storage season (1989), there was a significant difference between the foliar sprays treatment (retains 66,67% of its firmness) and the control (retains 61.77% of its firmness). At the end of the storage period in the first season there were significant differences between the three $CaCl_2$ treatments. Apples which were sprayed with $CaCl_2$ (5 g/L) retains 66.29% of its firmness at the harvesting date and 72.83% for those were dipped in 3% $CaCl_2$ and 85.55% for the vacuum infiltration treatment, while control retains 65.32%. At the end of the second storage season significant differences were observed between the dipping $CaCl_2$ treatment, the $CaCl_2$ vacuum infiltration and the control. No significant difference between the foliar spraying treatment and the control was observed. The obtained results are in agreement with those of many workers. Poovaiah (1986) reported that $CaCl_2$ dipping and/or vacuum infiltration improved maintaining fruit firmness during cold storage and the vacuum infiltration was more effective. Also Drake and Spayd (1983) and Laufmann et al. (1988) reported that Golden Delicious apples which were treated with $CaCl_2$ under pres-

Table 1

Effect of different calcium chloride treatments on fruit firmness (Lb/inch^2) of "Anna" apples during cold storage at 4 °C

Treatment	Seasons	Days in storage									
		0		15		30		40		50	
		1988	1989	1988	1989	1988	1989	1988	1989	1988	1989
Control		11.53	11.87	10.50	10.47	9.60	8.73	8.20	7.33	7.53	7.33
$CaCl_2$ 0.5% (3 sprays)		11.87	12.00	10.40	11.07	9.27	8.90	8.47	8.00	7.87	8.00
$CaCl_2$ 3% dipping		11.53	11.87	11.20	10.83	9.80	9.73	9.13	8.33	8.40	8.33
$CaCl_2$ 3% vacuum infiltration (350 mbar)		11.53	11.87	11.40	11.73	10.87	10.53	10.20	9.17	9.87	9.17
LSD 5%		N.S.	N.S.	0.729	N.S.	0.361	0.922	0.486	1.153	0.377	0.421
1%		N.S.	N.S.	1.055	N.S.	0.512	1.334	0.703	1.668	0.545	0.609

sure were firmer than untreated fruits. Batts and Bramlage (1977), Blanpied (1981), Lee and Dewey (1981) and Attia (1986) have indicated that dipping in $CaCl_2$ increased fruit firmness and delay fruit senescence. Bangerth et al. (1972) showed that firmness of Jonathan apples increased by 0.45 kg/cm^2 with dipping in $CaCl_2$.

An explanation for the difference between $CaCl_2$ dipping treatment and vacuum infiltration treatment was reported by Conway and Sams (1983). They indicated that the calcium content of fruits which dipped in $CaCl_2$ under vacuum were twice in calcium content compared with dipping in the same solution without vacuum. The non-significant results with spraying treatment could not confirmed by Raese (1988), who reported that different spray materials improved fruit firmness.

On the other hand, Stahly (1986) indicated that the time of application of calcium sprays affects fruit calcium content and fruit pitting.

Respiration rate

Data in Table 2 show the changes in respiration rates during the storage periods 1988 and 1989. There was an increment in respiration rate in all $CaCl_2$ treatments, as well as control with time in storage. Data show the effect of $CaCl_2$ treatments on reducing the respiration rate, when compared with control, especially at the end of the storage period. The lowest respiration rate was observed when used $CaCl_2$ with vacuum infiltration as a post-harvest treatment in both storage seasons followed by $CaCl_2$ post-harvest dipping treatment. The highest respiration rate was observed in the control. The differences between $CaCl_2$ spray treatment and the control was not significant in both seasons, except after two weeks in the second season. Also the significant difference was observed between $CaCl_2$ treatment and $CaCl_2$ vacuum infiltration treatment, as well as between them and control. Several workers have indicated an inverse relationship between calcium

Table 2

Effect of different calcium chloride treatments on the respiration rate (mg Co_2/kg/h) of "Anna" apples during cold storage at 4 °C

Treatment	Seasons	Days in storage									
		0		15		30		40		50	
		1988	1989	1988	1989	1988	1989	1988	1989	1988	1989
Control		16.87	15.84	29.10	26.53	36.77	31.13	40.40	39.07	50.07	43.77
$CaCl_2$ 0.5% (3 sprays)		16.60	11.73	29.00	24.30	36.47	31.40	40.26	38.80	49.47	41.23
$CaCl_2$ 3% dipping		16.87	15.84	20.27	22.57	32.57	28.07	33.33	34.00	37.17	36.17
$CaCl_2$ 3% vacuum infiltration (350 mbar)		16.87	15.84	18.97	20.67	29.57	24.77	33.83	27.80	34.73	31.47
LSD	5%	N.S.	N.S.	0.521	1.726	0.700	2.107	0.339	2.652	1.299	2.815
	1%	N.S.	N.S.	0.754	2.496	1.013	3.047	0.491	3.835	1.878	4.072

content and respiration in apples (Faust and Shear, 1972; Faust, 1974). Similarly, Bromlage et al. (1974) reported that a decrease in postclimacteric respiration as a peel calcium level increased from 400 to 300 ppm in Baldwin apples. Also Attia (1986), Chopro and Naqash (1986) reported a reduced rate of respiration as apple fruits were dipped in $CaCl_2$. Otherwise, Carl and William (1984) concluded that the respiration rate of "Golden Delicious" apples was not influenced by $CaCl_2$ infiltrated up to 12%, which supports the obtained results.

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Plant genetics and breeding

THE IMPACT OF GENOTYPE-ENVIRONMENTAL INTERACTION ON THE DETERMINATION OF THE COMPONENTS OF VARIATION AND OTHER GENETIC PARAMETERS IN MAIZE

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Twenty-five maize (*Zea mays* L.) varieties were evaluated under the environments of early sowing with narrow spacing, early sowing with broad spacing, late sowing with narrow spacing and late sowing with broad spacing, in order to study the nature of response of the components of variation and other genetic parameters to genotype × environmental interaction. The evidence indicates the occurrence of genotype × environmental interaction. In general, the genetic variances in respect to grain yield, and five other component characters, became reduced after the elimination of the interaction component from the total variance. The relative importance of the genotype × environmental interaction in determining these genetic parameters, and the need for their elimination in order to arrive at precise heritability estimates, are discussed.

Keywords: *Zea mays*, genotype × environmental interaction, genetic parameters

Introduction

The components of variation and other genetic parameters assume greater influence in the context of crop improvement, because any programme of breeding for high yield depends upon the nature and the magnitude of the variability present in the material, and the extent to which it is heritable. It is, therefore, imperative to partition the overall variability into its heritable and non-heritable components with the help of suitable genetic parameters. These parameters enable the prediction of the likely genetic gain through breeding. The accuracy, however, of such prediction would depend on the extent of stability with which these parameters respond to environments to which they are exposed. This is, presumably, an implication of the fact that these estimates are much influenced by the genotype —environmental interactions, and consequently do not reflect the true values. To ensure accuracy, under such circumstances, it is advisable to eliminate the interaction component from the total variance before estimating the genetic components of variance. It is with this objective that 25 maize varieties were evaluated under four environments and the results are discussed here.

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Materials and methods

The 25 maize varieties collected from the International Maize and Wheat Improvement Center, Mexico; Indian Agricultural Research Institute, New Delhi, India; and Bangladesh Agricultural Research Institute, Joydebpur, Bangladesh, were grown under four environments in a randomized block design with three replications. The varieties include Comp. Hunius, Across 7729, Savar-2, J-1, Ferke (II) 7539, JC-2, Chequisacha (I) 7833, Pantnoyar (I) 8033, Tlaltizapan 8033, Chequisacha 7845, Obregon 8045, Antalya (I) 8045, Obregon 8046, Adapazari 7948, Tlaltizapan 7948, Sadaf, Lamaquina 7827, Obregon 7748, Poza Rica 7737, Alazuala 7725, Arnd (I) 8156, Across 8156, Khaibhutta, Barnali and Shuvra. All four trials were conducted in the Bangladesh Agricultural University Farm, Mymensingh, Bangladesh, during the winter season of 1987—1988. The environments were created by varying the planting density (8.89 and 5.33 plants/m^2). The experiments were repeated over two sowing dates (2.12.87 and 12.1.88). The four environments were thus designated as E1 (early sowing, narrow spacing), E2 (early sowing, broad spacing), E3 (late sowing, narrow spacing) and E4 (late sowing, broad spacing). Each experimental plot consisted of single row five meters long, with a row to row spacing of 75 cm. Each experimental area was bordered on either side by two rows of the maize variety Barnali. All the treatments were uniformly fertilized and cultivated. A total of 10 plants were randomly selected from each variety (replication) environment for detailed observation of six characters, viz., grain yield per plant (g), ear length (cm), ear diameter (cm), kernel rows per ear, number of kernels per row and 1000-kernel weight (g).

Mean data were subjected to analysis of variance. The mixed model (a-varieties fixed and b-environments random) in its linear form was adopted in estimating components of variance. The combined (pooled) analysis of variance over environments was carried out following Singh and Chaudhary (1979). Genotypic and phenotypic variances and coefficients of variation were estimated following Burton (1952). The broad sense heritability and genetic advance at 5% selection intensity were computed according to Hanson et al. (1956) and Johnson et al. (1955).

Results and discussion

The combined analysis of variance (Table 1) indicated the differential influences of the environments on the development of grain yield, ear length, ear diameter and 1000-kernel weight through the significant variances due to environments. The environmental effect was, however, insignificant on kernel rows per ear and number of kernels per row. The variances due to genotype × environmental interaction were also significant for all traits, except ear diameter. It indicated that the

Table 1

Analysis of variance pooled over environments

Source	d.f.	Mean squares					
		Grain yield	Ear length	Ear diameter	Kernel rows/ear	No. of kernels/row	1000-kernel weight
Environments(E)	3	10461.07**	36.64**	4.65**	1.63	11.88	66927.67**
Genotypes (G)	24	1575.86**	5.92*	0.41**	2.50*	31.51*	7079.71**
GXE	72	734.53**	3.66**	0.13	1.31**	16.54**	2218.39**
Replications in environments	8	256.23	0.43	0.11	0.16	1.46	770.71
Error	192	242.97	0.99	0.09	0.36	1.20	645.35

*, ** Significant at 5% and 1% level, respectively.

Table 2

Components of variance and genetic parameters in E1

Character	Mean ± S.E.	Variance		Coefficient of variation		Broad sense heritability (%)	Genetic advance	Genetic advance in % of mean
		Phenotypic	Genotypic	Phenotypic	Genotypic			
Grain yield	95.45 ± 10.08	498.11	193.52	23.38	14.57	38.85	17.86	18.71
Ear length	14.00 ± 0.54	2.81	1.93	11.99	9.91	64.42	2.37	16.89
Ear diameter	3.85 ± 0.16	0.12	0.05	9.13	5.52	36.51	0.26	6.87
Kernel rows/ear	13.13 ± 0.40	1.11	0.64	8.01	6.07	57.54	1.25	9.49
No. of kernels/row	28.69 ± 0.50	7.49	6.74	9.45	9.05	89.88	5.07	17.67
1000-kernel weight	255.41 ± 11.65	1045.01	637.77	12.66	9.89	61.03	40.64	15.91

Table 3

Components of variance and genetic parameters in E2

Character	Mean ± S.E.	Variance		Coefficient of variation		Broad sense heritability (%)	Genetic advance	Genetic advance in % of mean
		Phenotypic	Genotypic	Phenotypic	Genotypic			
Grain yield	94.91 ± 10.37	528.23	205.66	24.22	15.11	38.93	18.43	19.42
Ear length	12.74 ± 0.49	1.24	0.51	8.74	5.63	41.51	0.95	7.47
Ear diameter	4.19 ± 0.15	0.10	0.03	7.47	4.28	32.55	0.21	5.01
Kernel rows/ear	12.81 ± 0.37	0.53	0.12	5.68	2.68	22.39	0.34	2.62
No. of kernels/row	29.10 ± 0.54	7.60	6.74	9.47	8.92	88.65	5.03	17.30
1000-kernel weight	258.96 ± 10.96	1084.50	724.43	12.72	10.39	66.79	45.32	17.49

varieties were not homogeneous in their response to environmental change for grain yield and most of its components.

Individual analyses (Tables 2, 3, 4 and 5) indicated higher grain yield and 1000-grain weight in early sowing than in late sowing. The highest phenotypic and genotypic coefficients of variation for grain yield was observed in all four environments, followed by 1000-kernel weight. These two traits also showed more coefficients of variation in early sowing than that of late sowing. The coefficients of variation for ear length and kernel rows per ear were higher in narrow spacing than

Table 4

Components of variance and genetic parameters in E3

Character	Mean ± S.E.	Variance		Coefficient of variation		Broad sense heritability (%)	Genetic advance	Genetic advance in % of mean
		Phenotypic	Genotypic	Phenotypic	Genotypic			
Grain yield	71.09 ± 9.05	425.96	180.43	29.03	18.89	42.36	18.01	25.33
Ear length	12.73 ± 0.45	2.06	1.45	11.27	9.45	70.26	2.08	16.31
Ear diameter	3.62 ± 0.20	0.15	0.04	10.78	5.26	23.75	0.19	5.28
Kernel rows/ear	12.89 ± 0.26	0.85	0.65	7.15	6.24	76.31	1.45	11.23
No. of kernels/row	28.39 ± 0.71	11.32	9.81	11.85	11.03	86.66	6.01	21.16
1000-kernel weight	204.13 ± 18.07	1612.95	632.93	19.67	12.32	39.24	32.47	15.90

Table 5

Components of variance and genetic parameters in E4

Character	Mean ± S.E.	Variance		Coefficient of variation		Broad sense heritability (%)	Genetic advance	Genetic advance in % of mean
		Phenotypic	Genotypic	Phenotypic	Genotypic			
Grain yield	80.67 ± 5.75	455.43	356.24	26.46	23.39	78.22	34.39	42.63
Ear length	13.89 ± 0.44	1.81	1.22	9.69	7.96	67.39	1.87	13.46
Ear diameter	3.72 ± 0.20	0.16	0.04	10.71	5.15	23.13	0.19	5.11
Kernel rows/ear	13.05 ± 0.36	0.64	0.25	6.11	3.81	38.42	0.64	4.89
No. of kernels/row	29.28 ± 0.75	3.83	2.15	6.68	5.01	56.17	4.43	15.12
1000-kernel weight	206.97 ± 16.67	2556.79	1722.69	24.43	20.05	67.38	70.18	33.91

in broad spacing. The rest of the characters showed variable coefficients of variability in different environments. Variable heritability values were also exhibited in different environments by all characters, except number of kernels per row, which showed moderate to high heritability in all environments. The heritability values for grain yield were higher in late sowing than that of early sowing. The reverse was, however, true for ear diameter. Kernel rows per ear showed moderate to high heritability values in narrow spacing and low heritability in broad spacing. The genetic advance in percentage of mean was highest for grain yield in the four environments, but the values were higher in early than in late sowing. Ear length

Table 6

Components of variance and genetic parameters in combined analysis

Character	Mean ± S.E.	Variance		Coefficient of variation		Broad sense heritability (%)	Genetic advance	Genetic advance in % of mean
		Phenotypic	Genotypic	Phenotypic	Genotypic			
Grain yield	85.53	313.08	70.11	20.69	9.79	22.39	8.16	9.54
	±9.00	476.93	233.96	25.77	17.99	49.59	22.17	26.52
Ear length	13.34	1.09	0.11	7.83	2.43	9.64	0.21	1.56
	±0.57	1.98	1.28	10.42	8.24	61.90	1.82	13.53
Ear diameter	3.85	0.12	0.02	9.08	3.96	18.99	0.14	3.55
	±0.18	0.13	0.04	9.52	5.05	28.99	0.21	5.57
Kernel rows/ear	12.97	0.37	0.01	5.24	2.45	21.52	0.30	2.33
	±0.35	0.78	0.42	6.74	4.70	48.67	0.92	7.06
No. of kernels/row	28.86	2.45	1.25	5.22	3.87	50.95	2.57	8.90
	±0.63	7.56	6.36	9.36	8.50	80.34	5.14	17.81
1000-kernel weight	231.37	1050.46	409.11	14.01	8.69	38.56	25.75	11.13
	±14.67	1574.81	929.46	17.37	13.16	58.61	47.15	20.80

Underlined figures indicate the mean over four environments.

and kernel rows per ear showed lower values of genetic gain in broad than in narrow spacing. The reverse was true for 1000-kernel weight.

Components of variance were calculated after eliminating the genotype × environmental interaction from the total variance and compared with the average of the four environmental means (Table 6). Indeed, there was a general drop in these values after accounting for the interaction component, but such a drop varied in degree between the component characters. The genotypic and phenotypic coefficients of variability were highest for grain yield in combined analysis, followed by 1000-kernel weight. These values were low for the other traits.

Similarly, the broad sense heritability values and the predicted genetic advance were calculated after eliminating the interaction component. Clearly, these two parameters were affected by the interaction, although to a varying magnitude (Table 6), for these values in respect to all characters were invariably lower than the four environmental means. The maximum heritability was characterized by the number of kernels per row, followed by 1000-kernel weight in the combined analysis. However, it was low with grain yield, ear diameter and kernel rows per ear; and was abnormally low with ear length in the pooled analysis. The most affected character by the genotype × environmental interaction was ear length, followed by kernel rows per ear and grain yield. The remaining three characters were comparatively less affected by the interaction.

As for predicted genetic advance, variations could be found between the characters not only in respect to the percentage of gain but also to the magnitude of the effect of interaction. The highest genetic advance was predicted for 1000-kernel weight followed by grain yield and number of kernels per row in the combined

analysis, but the least genetic advance was with ear length, followed by kernel rows per ear and ear diameter in the pooled analysis. Among all traits, ear length was subjected to the greatest influence of the genotype × environmental interaction, and ear diameter was least influenced by the interaction.

Results from the present study indicate that the heritability estimates and the values in respect of predicted genetic advance relating to all characters decreased after accounting for the genotype × environmental interaction. Presumably, therefore, the components of variation and the heritability estimated, based on the individual environments, were very much biased. Thus, if still more accurate predictions are intended, it is necessary to evaluate the genotypes, as suggested by Comstock and Robinson (1952), Johnson et al. (1955), Hanson et al. (1956) and Nei (1960), under a wider range of environments. The environmental factors involved in the present study were only the dates of sowing (early and late) and the spacing (narrow and broad). It may be indicated that, although the date of sowing and the spacing accounted for the significant amount of variation, as in this experiment, the probable impact of the such overall environmental factors as years and locations cannot be ignored. Singh and Atwal (1966) have shown in their studies that the contribution by years to variance was not so much as by locations and sowing dates. If so, it would be advantageous that the pace of progress could be quickened by incorporating all the possible environmental factors to which the varieties are desired to be exposed in a reasonably shorter period of time, irrespect of the number of years.

The significant outcome of this study, however, was that the number of kernels per row had indicated highest heritability values accompanied by moderate genetic gain, even after eliminating the interaction effects. If this were to be a meaningful combination as advocated by Johnson et al. (1955), a suggestion could be offered to exercise selection for this trait with a view to advance the structure of grain yield in maize.

Summary

Twenty five maize (*Zea mays* L.) varieties were evaluated under the environments of early sowing with narrow spacing, early sowing with broad spacing, late sowing with narrow spacing and late sowing with broad spacing, in order to study the nature of response of the components of variation and other genetic parameters to genotype × environmental interaction. Differential influences of the environments on the development of grain yield, ear length, ear diameter and 1000-kernel weight were observed. The evidence indicates the occurrence of genotype × environmental interaction for grain yield and four of its component characters. There was a general drop in the values of genetic variance after elimination of genotype × environmental interaction for grain yield, ear length, ear diameter, kernel rows per ear, number of kernels per row and 1000-kernel weight. The broad sense heritability and genetic advance in respect to all these traits also became reduced after the elimination of the interaction component from the total variance. The number of kernels per row indicated the highest heritability value accompanied by moderate genetic gain, even after elimination of the interaction effect. It is suggested to exercise selection for this trait with a view to advance the structure of grain yield in maize.

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Appendix — I

Mean values for various characters in maize varieties in E1

Varieties	Grain yield (g)	Ear length (cm)	Ear diameter (cm)	Kernel rows/ear	No. of kernels/row	1000-kernel weight (g)
Comp. Hunius	77.46	12.18	3.71	13.26	27.00	218.27
Across 7729	100.15	12.45	4.07	12.93	28.67	274.70
Savar-2	100.09	14.65	3.88	12.80	30.23	256.67
J-1	90.97	13.74	3.73	12.22	31.60	228.90
Ferke (II) 7539	96.84	14.18	3.65	12.78	30.86	242.42
JC-2	92.59	13.77	3.73	13.00	29.33	244.13
Chequisacha (I) 7833	88.52	13.06	3.80	12.62	25.17	279.53
Pantnoyar (I) 8033	90.37	12.95	3.66	14.03	24.76	260.17
Tlaltizapan 8033	83.22	13.13	3.82	14.47	23.77	245.03
Chequisacha 7845	73.61	12.51	3.91	11.80	26.13	244.87
Obregon 8045	91.02	12.75	4.05	12.68	26.73	269.83
Antalya (I) 8045	70.04	13.85	3.51	12.02	28.50	222.20
Obregon 8046	99.62	14.02	3.67	12.44	29.57	281.73
Adapazari 7948	96.04	13.87	3.96	12.11	29.17	273.00
Tlaltizapan 7948	104.35	15.35	3.59	12.15	32.17	258.67
Sadaf	104.50	14.90	3.69	13.68	32.37	255.33
Lamaquina 7827	119.73	15.51	4.43	13.38	29.63	294.00
Obregon 7748	84.37	12.88	4.05	14.75	26.24	223.33
Poza Rica 7737	105.24	14.85	4.23	14.95	29.00	246.00
Alazuala 7725	100.54	15.37	3.67	13.60	28.17	281.50
Arnd (I) 8156	121.34	14.75	3.74	13.93	35.33	266.00
Across 8156	70.25	13.67	3.51	13.33	28.44	202.07
Khaibhutta	69.92	12.82	3.56	12.22	26.25	211.50
Barnali	116.71	16.23	4.35	14.24	28.17	296.67
Shuvra	138.97	16.61	4.35	14.78	30.06	305.90
C.D. at 5%	28.68	1.55	0.46	1.13	1.43	33.16

Appendix — II

Mean values for various characters in maize varieties in E2

Varieties	Grain yield (g)	Ear length (cm)	Ear diameter (cm)	Kernel rows/ear	No. of kernels/row	1000-kernel weight (g)
Comp. Hunius	86.91	11.65	4.36	13.40	23.33	266.17
Across 7729	126.46	14.07	4.33	13.37	33.33	281.17
Savar-2	85.09	11.90	3.98	12.53	30.33	225.33
J-1	100.51	13.56	4.45	13.13	32.17	239.69
Ferke (II) 7539	94.16	12.30	3.91	11.90	26.83	248.13
JC-2	100.63	13.16	4.47	12.80	32.76	269.80
Chequisacha (I) 7833	98.23	12.23	4.23	12.70	27.73	287.00
Pantnoyar (I) 8033	105.99	13.59	4.33	13.20	30.73	269.17
Tlaltizapan 8033	106.17	13.47	4.29	13.47	27.00	282.50
Chequisacha 7845	91.34	13.45	4.08	12.05	29.00	267.03
Obregon 8045	92.09	12.37	4.01	12.40	27.73	267.33
Antalya (I) 8045	66.23	11.47	3.85	12.33	26.50	221.33
Obregon 8046	83.56	12.92	4.16	12.27	27.87	241.00
Adapazari 7948	100.58	12.67	4.05	12.57	29.33	265.23
Tlaltizapan 7948	113.38	14.06	4.16	12.73	32.67	287.17
Sadaf	74.55	11.27	4.07	13.75	22.50	220.83
Lamaquina 7827	113.93	13.08	4.62	12.73	22.50	307.17
Obregon 7748	117.09	13.93	4.46	13.40	31.33	281.83
Poza Rica 7737	102.87	13.39	4.33	12.67	29.00	285.50
Alazuala 7725	104.97	13.17	4.38	12.87	30.00	270.17
Arnd (I) 8156	84.63	12.13	3.93	13.00	31.00	233.17
Across 8156	44.36	11.14	4.09	13.08	28.17	215.00
Khaibhutta	74.68	12.13	3.62	13.33	30.00	185.50
Barnali	108.60	13.30	4.36	11.90	30.23	281.67
Shuvra	95.66	11.90	4.30	12.65	28.33	274.58
C.D. at 5%	29.51	1.39	0.42	1.05	1.52	31.18

Appendix — III

Mean values for various characters in maize varieties in E3

Varieties	Grain yield (g)	Ear length (cm)	Ear diameter (cm)	Kernel rows/ear	No. of kernels/row	1000-kernel weight (g)
Comp. Hunius	33.04	11.56	2.87	12.78	22.33	126.00
Across 7729	52.69	11.15	3.45	10.61	29.27	183.00
Savar-2	81.56	13.65	3.88	13.67	25.83	232.73
J-1	83.16	12.20	3.67	12.18	29.47	220.20
Ferke (II) 7539	75.16	12.55	4.07	14.25	27.60	237.33
JC-2	85.35	12.65	3.93	13.06	25.87	250.00
Chequisacha (I) 7833	65.48	11.36	3.35	11.67	26.40	222.33
Pantnoyar (I) 8033	68.58	12.01	3.78	12.93	24.30	216.67
Tlaltizapan 8033	62.21	12.91	3.67	12.88	28.00	192.33
Chequisacha 7845	81.77	15.47	3.80	13.33	28.83	197.44
Obregon 8045	76.73	12.64	3.70	13.49	30.13	198.33
Antalya (I) 8045	68.66	13.62	3.57	12.16	28.67	208.10
Obregon 8046	88.33	14.68	3.75	13.56	28.90	229.83
Adapazari 7948	94.59	13.24	3.90	12.52	28.43	218.00
Tlaltizapan 7948	71.68	12.48	3.39	11.92	31.10	229.00
Sadaf	75.37	13.95	3.73	12.28	30.23	221.08
Lamaquina 7827	38.78	9.12	3.52	13.75	19.50	182.67
Obregon 7748	91.66	14.20	3.85	13.60	29.44	243.10
Poza Rica 7737	78.01	13.04	3.82	13.12	27.17	209.50
Alazuala 7725	83.11	12.66	3.42	11.97	33.55	176.83
Arnd (I) 8156	74.07	13.28	3.49	13.03	34.33	163.25
Across 8156	42.98	12.16	3.60	13.75	30.00	163.83
Khaibhutta	65.47	12.09	3.05	12.83	31.23	155.67
Barnali	83.57	13.25	3.78	13.28	30.80	273.07
Shuvra	55.26	11.45	3.47	13.90	28.50	188.83
C.D. at 5%	25.75	1.29	0.56	0.74	2.02	51.44

Appendix — IV

Mean values for various characters in maize varieties in E4

Varieties	Grain yield (g)	Ear length (cm)	Ear diameter (cm)	Kernel rows/ear	No. of kernels/row	1000-kernel weight (g)
Comp. Hunius	82.32	13.07	3.37	13.00	29.25	186.67
Across 7729	66.54	14.32	3.87	13.40	30.36	160.43
Savar-2	82.80	14.35	3.73	11.83	29.61	253.37
J-1	91.69	13.75	3.77	13.67	30.40	221.87
Ferke (II) 7539	109.55	15.33	3.87	13.72	30.29	251.67
JC-2	83.99	13.75	3.98	12.17	27.53	256.33
Chequisacha (I) 7833	108.76	15.32	3.88	13.83	30.25	251.33
Pantnoyar (I) 8033	110.00	14.50	3.92	13.43	29.65	264.33
Tlaltizapan 8033	80.55	12.54	3.82	12.69	26.71	246.25
Chequisacha 7845	71.39	14.61	3.49	13.11	26.77	172.33
Obregon 8045	117.88	13.37	3.81	12.87	31.29	239.23
Antalya (I) 8045	72.27	13.52	3.82	13.42	30.35	185.37
Obregon 8046	85.99	14.48	3.46	12.95	27.67	261.60
Adapazari 7948	99.76	14.09	3.84	12.28	31.52	240.17
Tlaltizapan 7948	84.85	14.26	3.71	12.53	32.33	219.50
Sadaf	61.74	11.73	3.66	12.42	28.48	169.50
Lamaquina 7827	67.22	12.17	4.01	13.88	27.70	178.33
Obregon 7748	84.94	13.42	3.84	13.57	28.91	201.07
Poza Rica 7737	65.15	12.83	3.69	13.00	30.44	162.33
Alazuala 7725	89.44	15.62	3.98	13.65	25.48	258.17
Arnd (I) 8156	57.16	13.80	3.99	13.00	30.21	147.60
Across 8156	53.18	12.55	3.42	12.92	29.28	145.00
Khaibhutta	36.64	12.00	2.69	12.58	28.00	110.67
Barnali	90.66	15.25	3.77	14.17	29.62	219.13
Shuvra	62.08	14.49	3.68	12.22	30.03	172.07
C.D. at 5%	16.37	1.26	0.57	1.02	2.13	47.45

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ANALYSIS OF RECIPROCAL DIFFERENCES IN INDIAN MUSTARD

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An analysis of reciprocal cross differences in a 12-parent diallel mating design was conducted on eight characters in Indian mustard (*Brassica juncea* L.—Czern and Coss). General combining ability effects predominated in all the characters studied. Reciprocal cross effects were evident and more pronounced than were specific combining ability effects, though the latter were also significant for all the traits studied. A comparision of selected crosses, based on high specific combining ability and reciprocal effects, indicated *vice versa* differences in cross effects for the majority of characters. Only one cross P5 × P11 (B.I.C. 1382 × B.I.C. 1702) showed reciprocal cross effects in the favourable direction for all the eight characters. It is assumed that the observed differences in reciprocal cross effects originated from cytoplasmic diversity in the base material.

Keywords: *Brassica juncea*, mustard, reciprocal differences, combining ability.

Introduction

It is widely known that, besides nuclear genes, maternal cytoplasm also influences the character expression of the progeny. Breeders generally ignore this fact in their crop improvement programmes. Recent reports (Kalsy and Sharma, 1972; Singh, 1973; Yavada et al., 1974; Buson, 1980; Singh and Singh, 1981) suggest that greater success could be achieved in selection if reciprocal differences are given due weight. The present paper draws attention to such reciprocal differences, in particular the possible cause of this interaction and the necessary breeding procedure in order to exploit the observed nature of gene action for the quantitative traits in Indian brown mustard.

Materials and methods

Twelve diverse lines (namely, BIC-1047, BIC-1114, BIC-1221, BIC-1323, BIC-1382, BIC-1427, BIC-1439, BIC-1534, BIC-1585, BIC-1624, BIC-1702 and BIC-1648 of brown mustard, chosen from different agroclimatic conditions of India, were crossed in a diallel mating design, including reciprocals. The 132 F_1s thus obtained and their parental selfs were studied in a randomised block design with three replications of two tiers each. The spacings were 45 cm between rows and 15 cm between plants. The recommended agronomic practices were adopted. Non-experimental rows were planted all round the experiment to avoid any border effects. Five random plants were tagged from each plot in the early stages, and were observed for eight characters; days to flower, primary and secondary branches, plant height (cm), main shoot length (cm), siliquae on main fruiting shoot, seeds per siliqua and seed yield per plant (g). The data were analysed following Griffing (1956) Model I, Method I.

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Results

A comparison of means and ranges of variation of plants with hybrids (both F_1s and reciprocal included) indicated higher means and larger variation for hybrids for all the eight characters (Table 1). The mean values for reciprocal F_1s were higher than the corresponding F_1s for such characters as days to flower, number of primary and secondary branches, plant height and seed yield per plant,

Table 1

Range of variation, mean values and analysis of variance for eight characters in a diallel cross in Indian mustard

Source		Days to flowering	Primary branches	Secondary branches	Plant height
Range of variation					
Parents		40.9—58.7 (45.7)	5.1—10.1 (8.1)	17.7—27.0 (18.9)	112.7—221.7 (169.6)
F_1s		40.1—64.9 (46.4)	5.2—13.1 (8.5)	19.1—29.9 (20.6)	115.5—230.6 (171.1)
Reciprocal F_1s		38.1—76.7 (54.2)	6.7—11.9 (9.0)	17.9—41.5 (28.8)	110.4—255.1 (199.9)
ANOVA	D.F.		Mean sum of squares		
Rep.	2	0.5	34.5	441.2	12820.0
Treat.	143	698.7**	20.4**	142.9**	13564.4**
Rep. X Treat.	286	94.1**	3.3**	29.9**	667.5**
Error	1728	56.7	2.9	24.8	268.8

Source		Main shoot length	Siliquae on main shoot	Seeds/siliqua	Seed yield/plant
Range of variation					
Parents		45.3—61.5 (53.4)	35.6—51.3 (43.4)	12.7—15.2 (13.6)	11.2—29.5 (15.5)
F_1s		50.2—68.5 (62.4)	38.8—62.0 (52.5)	12.3—15.6 (14.5)	12.0—34.0 (20.5)
Reciprocal F_1s		40.9—72.6 (55.8)	35.9—52.1 (45.4)	12.0—18.4 (13.8)	15.7—44.7 (25.6)
ANOVA	D.F.		Mean sum of squares		
Rep.	2	3686.3	1366.1	81.7	834.5
Treat.	143	415.3**	567.0**	12.5**	1267.1**
Rep. X Treat.	286	223.8**	269.8**	6.6**	138.8**
Error	1728	103.1	87.9	2.5	54.9

Figures in parentheses indicate means.
(**) Significant at p = 0.01.
Rep. = Replications.
Treat. = Treatments.

while the reverse was true for the rest of the characters. Reciprocal F_1s were observed having larger ranges of variation than the F_1s for all characters except primary branches and siliquae on the main shoot. The analysis of variance (Table 1) revealed further significant differences among the treatments (parents and crosses together) for all the characters.

The analysis of variance for combining ability (Table 2) showed that variation among the parents, due to differences in general combining ability (g.c.a.), among

Table 2

Analysis of variance for combining ability with reciprocal effects

Source	D.F.	Days to flowering	Primary branches	Secondary branches	Plant height	Main shoot length	Siliquae on main shoot	Seeds/siliqua	Seed yield/plant
g.c.a.	11	445.9**	10.5**	62.5**	8649.4**	59.8**	154.7**	2.2**	185.7**
s.c.a.	66	10.6**	0.5**	5.0**	217.3**	32.0**	39.7**	0.7**	41.6**
r.c.e.	66	21.0**	1.7**	12.2**	330.5**	19.6**	21.4**	0.7**	63.9**
Error	1728	3.7	0.2	1.7	17.9	6.9	5.7	0.2	5.5

(**) significant at p = 0.01.
g.c.a. = general combining ability.
s.c.a. = specific combining ability.
r.c.e. = reciprocal cross effects.

the crosses, due to differences in specific combining ability (s.c.a.), and among the reciprocals were significant for all the characters. The magnitude of variance due to reciprocal cross effects (r.c.e.) were more than the variance due to s.c.a. for such characters as days to flower, number of primary and secondary branches, plant height and seed yield per plant. It was, however, equal for seeds per siliqua and low for the remaining two characters; main shoot length and siliquae on the main shoot.

Significant reciprocal differences were shown by 17 crosses for days to flowering, 23 for primary branches, 21 for secondary branches, 34 for plant height, 13 for main shoot length, 18 for siliquae on the main shoot, 27 for seeds per siliqua and 22 for seed yield per plant. A comparison of some crosses selected on the basis of s.c.a. effects and r.c.e. (Table 3) revealed that the crosses which showed better s.c.a. for all the eight characters were P2 × P3, P3 × P11, P5 × P10 and P7 × P8; for the majority (5 to 7) of characters were P3 × P12, P4 × P11, P5 × P6, P1 × P4, P1 × P10 and P8 × P10, and for two to four characters were P2 × P9, P2 × P10, P2 × P12, P5 × P11, P8 × P9 and P8 × P11. Based on r.c.e. of these crosses, it was observed that three crosses, P2 × P10, P2 × P12 and P5 × P11 showed significant reciprocal effects for all the eight characters, eight crosses, P3 × P12, P5 × P6, P1 × P4, P1 × P10, P2 × P9, P8 × P9, P8 × P10 and P8 × P11 for the majority (5 to 7) of characters, and the remaining five crosses for three or four characters.

Table 3

Estimates of specific combining ability (s.c.a.) and reciprocal cross effects (r.c.e.) of some selected crosses in full diallel

Cross	Days to flowering	Primary branches	Secondary branches	Plant height	Main shoot length	Siliquae on main shoot	Seeds/siliqua	Seed yield/plant
P2 × P3	−0.7	0.7*	1.9*	0.3	1.0	1.8	0.1	14.8**
	(−0.4)	(−0.3)	(−0.5)	(−5.8*)	(−0.7)	(−1.8)	(−0.6*)	(−4.1*)
P3 × P11	−0.8	1.0**	1.8	14.9**	4.6**	9.5**	0.9	16.8**
	(2.0**)	(−0.2)	(0.1)	(13.8**)	(0.9)	(3.1)	(−0.7*)	(11.8**)
P3 × P12	−5.4**	0.6*	2.4**	22.8**	4.6**	4.3**	0.4	10.5**
	(−4.0**)	(−0.5)	(−0.6)	(−11.6**)	(5.6**)	(5.5**)	(0.2)	(12.5**)
P4 × P11	2.7*	0.7*	1.9*	7.9**	1.8	1.4	1.2**	14.5**
	(−0.6)	(−0.2)	(−0.2)	(10.6**)	(−0.5)	(0.5)	(−0.6*)	(6.3**)
P5 × P6	5.1**	1.0**	3.3**	23.6**	4.5**	10.5**	0.1	11.5**
	(3.5**)	(0.7*)	(1.2)	(16.5**)	(2.7)	(2.8)	(0.7*)	(13.5**)
P5 × P10	−1.8	0.6*	2.9**	4.3	2.7	0.9	0.9**	12.5**
	(−1.4)	(−0.4)	(−2.0*)	(−12.0**)	(−1.3)	(−1.3)	(0.8**)	(6.3*)
P7 × P8	−2.1	0.6*	2.2*	7.8**	1.9	4.4**	0.5*	8.9**
	0.0	(−0.4)	(1.8*)	(6.0*)	(−2.0)	(1.7)	(0.2)	(5.6**)
P1 × P4	1.5	0.5	1.3	24.6**	2.9	5.1**	−0.8**	4.0*
	(−0.8)	(−1.0**)	(−2.2*)	(−32.5**)	(−3.0)	(−5.5**)	(0.6*)	(−3.6*)
P1 × P10	3.6**	0.3	1.5	23.0**	2.3	1.5	0.3	15.00**
	(−6.0**)	(−0.8*)	(−1.4)	(−32.9**)	(1.4)	(−3.7*)	(1.9**)	(5.0**)
P2 × P9	0.6	0.5	2.6**	−1.8	−6.2**	−3.7*	−0.4	−3.9*
	(−3.5**)	(−1.2**)	(−0.5)	(−14.5**)	(1.7)	(3.9*)	(0.8**)	(6.3**)
P2 × P10	−1.5	−0.1	0.5	−3.6	3.2	2.2	−0.2	3.5*
	(−4.5**)	(−1.1**)	(−3.3**)	(−28.4**)	(−4.4*)	(−6.3**)	(0.7*)	(4.5**)
P2 × P12	−0.5	0.2	0.9	−1.9	−1.6	−1.3	0.3	3.5*
	(−5.4**)	(−0.7*)	(−1.8*)	(−26.3**)	(−4.7**)	(−7.6**)	(−1.0**)	(−6.5**)
P5 × P11	−4.5**	−0.7*	−1.9*	15.8**	−1.7	2.9	−0.5*	3.8*
	(−3.5**)	(1.3**)	(2.4**)	(13.0**)	(8.6**)	(7.7**)	(1.2**)	(14.5**)
P8 × P9	−1.8	−0.4	0.2	−1.3	2.7	−1.2	−1.1**	4.0*
	(−3.7**)	(−1.4**)	(−2.0*)	(−31.2**)	(0.7)	(−4.8**)	(−0.5*)	(−3.7*)
P8 × P10	2.6*	0.5	0.2	7.5**	3.1	−6.1**	−0.3	6.5**
	(−6.0**)	(−0.8**)	(−3.1**)	(−19.0**)	(1.8)	(−5.5**)	(0.0)	(−3.8*)
P8 × P11	0.7	0.1	0.2	−16.8**	−1.4	−2.2	−0.6*	4.0*
	(−0.7)	(−1.2**)	(−4.0**)	(−30.9**)	(−4.3*)	(−9.5**)	(0.0)	(−6.5**)

(**) Significant at p = 0.01, (*) Significant at p = 0.05.
Values in parentheses indicate reciprocal cross effects.
Parents:
P1 = BIC—1047, P2 = BIC—1114, P3 = BIC—1221, P4 = BIC—1323, P5 = BIC—1382, P6 = BIC—1427, P7 = BIC—1439, P8 = BIC—1534, P9 = BIC—1585, P10 = BIC—1624, P11 = BIC—1702, P12 = BIC—1648.

Discussion

The analysis of variance of combining ability suggested that both additive and non-additive gene effects were important in the inheritance of the characters studied. However, additive effects were more predominant, as g.c.a. variances were comparatively larger than s.c.a. variances, as also reported by Singh (1973), Yada-

va et al. (1974), Labana et al. (1975), Asthana and Pandey (1977), Anand and Rawat (1978) and Jindal and Labana (1986). Since these components are directly or indirectly related to seed yield (Rawat and Anand, 1977) and are governed by both additive and non-additive gene action, it may be worthwhile to utilise recurrent selection procedures in the segregating generations. Towards this objective a population should be developed based on parental lines which show superior *per se* performance, together with better combining ability and F_1 performance.

In the present study, the lines that showed these criteria included BIC-1624, BIC-1382, BIC-1439, BIC-1114 and BIC-1702. Therefore, it may be useful to create a heterogeneous population by crossing these five superior lines *inter se* before initiating random matings in F_2, to permit greater recombination. This is likely to break unfavourable linkages and confer a wide genetic base (Joshi and Dhawan, 1966). The estimates of reciprocal effects revealed significant differences among reciprocal crosses for all the characters, which were more pronounced for days to flowering, secondary branches, plant height, main shoot length, siliquae on the main shoot and seed yield per plant, and were less pronounced (although highly significant) for seeds per siliqua and primary branches. This indicated that nucleo-cytoplasmic interaction influenced the expression of these characters. Besides Indian brown mustard (Yadava et al., 1974; Asthana and Pandey, 1977; Jindal and Labana, 1986) and winter rape (Buson, 1980), reports on reciprocal cross differences for various characters are available in linseed (Anand and Murty, 1969), maize (Bhat and Dhawan, 1969; Kalsy and Sharma, 1972) and chilli (Singh and Singh, 1981). R.C.E. in Indian brown mustard for primary and secondary branches, plant height, length of main shoot, siliquae on the main shoot, seed weight and seed yield (Yadava et al., 1974; Asthana and Pandey, 1977; Jindal and Labana, 1986) and in winter rape for yield, leaf area and flowering (Buson, 1980) have been reported.

Considering together s.c.a. and r.c.e. of F_1s, it was observed that some crosses having better s.c.a. also had significant r.c.e. for one or more characters in a favourable direction. In such a situation the choice of proper female parent is likely to further enhance the best use of beneficial non-additive effects in a specific cross. In the present investigation only one cross P5 × P11 (BIC-1382 × BIC-1702) was found to possess the favourable r.c.e. for all the eight characters studied (Table 3). Incidentally, parents involved in this cross also had high g.c.a. for the majority of characters, thus making possible the exploitation of its double beneficial additive and non-additive effects. The remaining crosses either showed significantly negative r.c.e. for all, or for one or more, characters. Beneficial effects of reciprocal crossing in maize to exploit days to silking and grain yield were earlier reported by Kalsy and Sharma (1972) and Dhawan et al. (1966), respectively.

The observed reciprocal cross differences cannot be related to any universally recognised mechanism determining reciprocal differences. Since, in the process of fertilisation, very little is contributed by male cytoplasm, any differences that may arise in the reciprocal cross could be due to the difference in the cytoplasm of the maternal gamete. Cytoplasmic differences among varieties can arise due to several factors, chief among which are the geographical isolation of the ancestral genotypes followed by constant natural and/or human selection under varied environments,

and the origin of new genotypes through natural hybridization among existing compatible species, together with parallel environments in their evolutionary history. Both these factors might have contributed in the present case, as *B. juncea* has an emphidiploid origin (Morinaga, 1934) from the basic genomic species of *B. campestris* (n = 10, A genome) and *B. nigra* (n = 8, B genome). There are several known n = 10 species having genome A that freely cross among themselves. There are also a number of morphologically deviant types both in *B. nigra* and *B. campestris*. Presumably, these might have contributed different cytoplasm in the evolution of this amphidiploid species, whose natural habitat ranged from the Mediterranean to subtropical regions in South Asia and the Far East. Since, in the present study, the parental lines were chosen from widely adapted ecogeographical regions of India, it is possible that these lines have varied cytoplasmic backgrounds. Furthermore, constant selection pressure for yield and adaptability under varied environments might have induced variability in the cytoplasm. The role of parental diversity in the manifestation of quantitative differences for agronomic characters, as influenced by specific cytoplasmic effects in their crosses, has been suggested by Dhawan and Paliwal (1964), Kalsy and Sharma (1972) in maize, Anand and Murty (1969) in linseed. Durrant (1965) suggested that changes in genic behaviour could occur under different paternal and maternal genetic backgrounds, leading to reciprocal differences in linseed.

The present study thus suggests the importance of reciprocal cross effects in the manifestation of quantitative characters. It is, therefore, recommended that reciprocal effects in Indian mustard should not be ignored while selecting parents for plant improvement, especially when widely diverse types are included in the breeding programme.

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HALF-DIALLEL ANALYSIS OF DIFFERENT CHARACTERS IN WHEAT ANTHER CULTURE

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Half-diallel analysis of F_1 crosses among six inbred winter wheat cultivars was carried out for investigating the inheritance of regeneration ability in anther culture. General and specific combining abilities were estimated and found to be highly significant for all characters studied, except for embryoid induction frequency. However, the proportion due to GCA effects was much higher than SCA effects in all characters studied (responding anthers frequency, total number of embryoids per anther responded, green plantlet induction frequency and albino plantlet induction frequency). Of the 21 entries, the largest mean responding anthers (35.8) and green plantlet induction (23.38) were observed on anthers of Mv 18 × Mv 21, while the largest mean total number of embryoids per anther responded (3.95), and albino plantlet induction frequency (31.34) were observed using anthers of Mv 21 × Kaláka and F 2076 cultivar, respectively. The results suggested that additive genetic effects are more important than non-additive in determining the inheritance of the observed characters.

Keywords: wheat, anther culture, F_1-crosses, diallel-analysis

Introduction

The use of haploids in wheat breeding programs is an established technique today. An even wider applicability depends mainly on an increased reproducibility of the haploid induction during different seasons and for different genotypes, coupled with decreased costs for the procedure.

Techniques for *in vitro* androgenesis have recently been developed in many laboratories. Among agricultural species many cereals will give embryogenetic plants: barley (Kuhlmann and Wehr, 1989), triticale (Charmet and Bernard, 1984), wheat (Chu et al., 1990), rye (Thomas et al., 1975) and maize (401 Research Group, 1975; Petolino et al., 1988).

Genetic factors have been noted to be major contributors to *in vitro* growth responses of cultured plant tissues (Lazar et al., 1984b; Ou et al., 1989; Raguin, 1982). This has been of particular interest to plant breeders concerned with the utilization of *in vitro* techniques in the development of useful germplasm. In wheat, as in many other crops, applications of doubled haploid breeding are currently limited by the low frequency of plantlet production per anther (Bullock et al., 1982). Other limitations include variation in response frequencies, due to the anther donor plant environment and to genotype × environment interaction (Lazar et al., 1984a).

This study was designed to estimate general and specific effects and other parameters for embryoid production and subsequent plantlet development in anther cultures of wheat.

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Materials and methods

Half diallel crosses were made among six winter wheat cultivars adapted to Hungarian conditions. The cultivars used were the following: Mv 19, Mv 18, Mv 21, F 2076, Hana and Kaláka. The 15 F_1 hybrids and six parents were grown under field conditions. Twenty spikes from each entry were collected at middle to late uninucleate microspores stages. The optimal pollen stage was determined from spike and anther morphology. The spikes were removed from the flag leaf sheath and sterilized.

The experiment was designed as a randomized complete block with 21 genotypes and four replications. Each replicate included five spikes for each entry and one spike in one petri dish as one experimental unit.

The embryoid induction medium was used according to Quyang (1986) Potato 2 medium. The regeneration media was used according to Zhuang and Xu (1983) medium 190—2.

Anthers were taken from each spike and cultured per 5.5 cm plastic petri dish with embryoid induction medium (P_2) and incubated at 28 °C ± 2 °C, in darkness and humidity, in the incubator apparatus for 42 days. The total number of responding anthers and the total number of embryoids were recorded. Embryos were transferred to a regeneration medium (190—2) for shoot and root development, the cultures were then placed in controlled incubators under continuous white fluorescent light at 25 °C ± 2 °C for four weeks after transferring the embryos. Then the total number of green plantlets and the total number of albino plantlets were recorded.

The responding anther frequency character was measured by percentage based on the total number of anther responded, divided by the total number of anther cultured. The total number of embryoids per anther responded character was measured by percentage, based on the total number of embryoid, divided by the total number of anther responded, and the green plantlet induction frequency was measured by percentage based on the total number of green plantlets, divided by the total number of embryoids which were transferred to the regeneration medium. The albino plantlet frequency was measured by percentage based on the total number of albino plantlets, divided by the total number of embryoids which were transferred to the regeneration medium. These characters were measured for each entry.

Data were subjected to the analysis of variance according to Cochran and Cox (1963), and the following statistical genetic estimates were performed:

— Mid parents heterosis (H.MP %) was calculated as:

$$H\% = \frac{F_1 - M.P}{M.P} \times 100$$

— Better parents heterosis (H.BP %) was calculated as:

$$H\% = \frac{F_1 - B.P}{B.P} \times 100$$

— General combining ability (GCA) and specific combining ability (SCA) were partitioned from total genotypic variance, using Griffing's method 2, model 1 (Griffing, 1956).

The percentage data for all traits were transformed by using arcsin $x^{1/2}$ transformation, except for total number of embryoids per anther responded prior to statistical analysis.

Results

The results of the analysis of variance of all genotypes for all characters are presented in Table 1. Tests of significance of the mean squares of genotypes indicated the presence of highly significant difference among these genotypes. However, the mean squares of replications was significant at 0.05 level in the case of the total number of anthers responded, where the genotypic variance was almost ten times higher than the variance of replications.

The means of the six parents and their F_1 hybrids are presented for all traits in Table 2. Of the six parental lines, the greatest mean responding anthers

Table 1

The analyses of variance and the mean squares for all traits of the parents and F_1 hybrids

S.O.V.	d.f.	Responding anthers	Total number of embryoids/anther responded	Green plantlet induction	Albino plantlet induction
Genotype	20	222.24**	1.86**	112.73**	252.76**
RePs.	3	25.30*	0.51	16.26	12.37
Error	60	8.39	0.25	29.88	16.36

* significant at 0.05% level
** significant at 0.01% level

Note: the data were estimated from transformed values (arcsin $x^{1/2}$) except for total number of embryoids per anther responded.

Table 2

The means of the parents and their F_1 hybrids for all traits

Genotypes	Total number of anthers cultured	Responding anthers	Total number of embryoids/anther responded	Green plantlet induction	Albino plantlet induction
Mv 19	1355	31.06	3.33	22.77	14.16
Mv 18	1941	25.42	2.45	21.50	30.78
Mv 21	1767	21.91	3.40	19.73	23.15
F 2076	1785	20.16	2.71	16.93	31.34
Hana	1707	6.34	1.18	0.00	0.00
Kaláka	2242	25.62	2.62	23.32	26.18
Mv 19 × Mv 18	1622	26.03	2.22	19.54	22.46
Mv 19 × Mv 21	1893	28.52	2.56	22.69	13.77
Mv 19 × F 2076	1292	28.10	2.54	18.94	17.33
Mv 19 × Hana	1660	14.72	1.51	13.87	14.56
Mv 19 × Kaláka	1806	28.51	2.29	24.03	18.54
Mv 18 × Mv 21	2065	35.80	3.10	23.38	29.58
Mv 18 × F 2076	1264	29.78	2.66	19.71	24.37
Mv 18 × Hana	1647	15.32	1.51	20.93	17.06
Mv 18 × Kaláka	1897	28.95	2.63	23.22	26.76
Mv 21 × F 2076	1778	26.16	2.61	23.31	23.30
Mv 21 × Hana	2127	15.19	2.21	21.36	16.66
Mv 21 × Kaláka	1963	32.42	3.95	21.64	22.05
F 2076 × Hana	1719	14.66	2.30	16.25	23.45
F 2076 × Kaláka	1773	24.93	3.35	17.52	25.05
Hana × Kaláka	1917	15.78	1.75	15.34	4.98
LSD 0.05 %		4.098	0.700	7.732	5.721
0.01 %		5.448	0.931	10.280	7.606
0.001%		7.056	1.206	13.313	7.606

Note: the data were estimated from transformed values (arcsin $x^{1/2}$) except for total number of embryoids per anther responded.

Table 3

The estimates of heterosis based on the mid-parental (M.P) *and better parental* (B.P) *values for all traits*

	Responding anthers	Total number of embryoids/anther responded	Green plantlet induction	Albino plantlet induction
M.P	21.75	2.62	17.38	20.94
B.P	31.06 Mv 19	3.40 Mv 21	23.32 Kaláka	31.34 F 2076
F_1	24.32	2.48	20.12	19.99
H (F_1, M.P)%	11.81**	−5.34**	15.77**	−4.54**
H (F_1, B.P)%	−21.70**	−27.06	−13.72**	−36.22**
LSD (F_1, M.P.)				
0.05% =	1.413	0.244	2.667	1.973
0.01% =	1.903	0.328	3.591	2.657
LSD (F_1, B.P.)				
0.05% =	3.022	0.522	5.702	4.219
0.01% =	4.068	0.702	7.678	5.681

** Significant at 0.01% level

Note: the data were estimated from transformed values (arcsin $x^{1/2}$) except for total number of embryoids per anther responded.

frequencies were observed in Mv 19, Kaláka and Mv 18 (31.6%, 25.62% and 25.42% from anthers cultured, respectively). The lowest one was Hana, 6.34% from anthers cultured. The means showed that Mv 19, Mv 18 and Kaláka were superior for all traits, and Hana cultivar was generally the poorest for all traits. However, Mv 21 was the best parent for the total number of embryoids per anther responded. On the other hand, the greatest overall total number of embryoids per anther responded was observed in the cross Mv 21 × Kaláka.

Table 4

The half diallel crosses analyses of variance and the mean squares of the F_1 hybrids and their parents for all traits

S.O.V.	d.f.	Responding anthers	Total number of embryoids/anther responded	Green plantlet induction	Albino plantlet induction
GCA	5	314.20**	2.20	98.86**	260.06**
SCA	15	18.18**	0.286	21.36**	33.47**
Error	60	8.39	0.245	29.88	16.36
GCA : SCA	—	17.3 : 1	7.7 : 1	4.6 : 1	7.8 : 1

** Significant at 0.01% level

Note: the data were estimated from transformed values (arcsin $x^{1/2}$) except for total number of embryoids per anther responded.

Table 5

General combining ability effects of parental lines for all traits

Parents	Responding anthers	Total number of embryoids/anther responded	Green plantlet induction	Albino plantlet induction
	GCA	GCA	GCA	GCA
Mv. 19	2.261**	−0.094	0.092	−3.348**
Mv. 18	2.988**	−0.074	1.166	5.017**
Mv. 21	2.771**	0.470**	1.804	1.267**
F 2076	0.069	0.193	0.229	3.987**
Hana	−10.228**	−0.759**	−5.589**	−7.367**
Kaláka	2.139**	0.263*	2.297	0.442
S.E.	0.763	0.130	1.440	1.066

* Significant at 0.05% level
** Significant at 0.01% level
Note: the data were estimated from transformed values (arcsin $x^{1/2}$) except for total number of embryoids per anther responded.

The heterosis effects were determined for all characters, and the results are presented in Table 3. The results showed the presence of positive significant estimates of heterosis for responding anther frequency and green plantlet induction frequency, especially when averages of hybrids (F_1) were compared to the mid parents (M.P). On the other hand, the average means of hybrids did not significantly exceed the better parent (B.P) for all characters. However, it could be claimed that certain F_1 hybrids significantly exceed the better parent for responding anther frequency, total number of embryoids per anther responded and green plantlet induction, such as Mv 18 × Mv 21, Mv 21 × Kaláka and Mv 19 × Kaláka, respectively.

Mean squares from the half diallel crosses analysis are presented in Table 4. Tests of significance of the mean squares of general combining ability (GCA) and specific combining ability (SCA) showed that GCA and SCA were highly significant, except for the total number of embryoids per anther responded. However, the proportion due to GCA effects was much higher than SCA effects in all characters studied.

The estimates of GCA effects for each parental line and SCA effects for each cross on all the characters studied are shown in Tables 5 and 6, respectively. Positive values indicate a contribution towards responses, while negative values represent the opposite. Among the parental lines examined, only Hana had highly negative significant GCA effects on all the characters, the other five parental lines showed positive GCA effects in most characters. Especially Mv 18 × Mv 21 and Mv21 × Kaláka were the best combiners for the *in vitro* androgenesis response. However, Mv 19 cultivar had positive GCA value green plantlet induction and negative GCA value albino plantlet induction, showing that it was the best com-

Table 6

The specific combining ability for each cross for all traits

Crosses	Responding anthers	Total number of embryoids/anther responded	Green plantlet induction	Albino plantlet induction
Mv 19 × Mv 18	−3.1	−0.1	−1.9	0.6
Mv 19 × Mv 21	−0.4	−0.3	0.6	−4.3
Mv 19 × F 2076	1.9	−0.1	−1.6	−3.5
Mv 19 × Hana	−1.2	−0.1	−0.8	5.1*
Mv 19 × Kaláka	0.2	−0.4	1.4	1.3
Mv 18 × Mv 21	6.1**	0.2	0.2	3.1
Mv 18 × F 2076	2.8	0.0	−1.9	−4.8
Mv 18 × Hana	−1.3	−0.2	5.1	−0.7
Mv 18 × Kaláka	−0.1	−0.1	−0.5	1.1
Mv 21 × F 2076	−0.6	−0.6*	1.1	−2.1
Mv 21 × Hana	−1.2	0.0	4.9	2.6
Mv 21 × Kaláka	3.6*	0.7*	−2.7	0.2
F 2076 × Hana	0.9	0.4	1.4	6.7**
F 2076 × Kaláka	−1.2	0.4	4.8	0.5
Hana × Kaláka	−0.0	−0.3	−1.6	−8.2**
S.E.	1.741	0.297	3.285	2.430

* Significant at 0.05% level
** Significant at 0.01% level
Note: the data were estimated from transformed values (arcsin $x^{1/2}$) except for total number of embryoids per anther responded.

biner for the *in vitro* green plantlet induction. Generally, the magnitude and the sign of the GCA of each parental line and the SCA for each cross agree with our previous observations on their individual performance.

Discussion

The six parents for the diallel were chosen because of their differences in the characters under study. In the population under study, variations due to genotypes were the largest components of the total variability, and most of the genotypic variance was due to GCA effects, indicating that additive genetic variance was a primary contributor to the observed responses.

The importance of additive effects in anther culture responsiveness had also been suggested by a previous study (Lazar et al., 1984b) for the inheritance of regeneration frequency of anther cultures in a diallel cross of five spring wheat cultivars.

Similar results have been found in hexaploid triticale for embryogenesis and plant regeneration (Charmet and Bernard, 1984) in wheat for callus induction and plantlet regeneration (Chevrier et al., 1990), and in maize for shoot and root forming capacities (Bechert and Oing, 1984).

Not all of the variations among crosses were attributable to additivity, since SCA effects were also significant except for the total number of embryoids per anther responded in this study. Thus non-additive genetic effects (dominance and epistasis) may play a role in the expression of these characters. These results are similar to the previous reports (Lazar et al., 1984b; Charmet and Bernard, 1984).

Both GCA and SCA effects were significant in most characters, indicating that additive and non-additive genetic effects are important for the characters examined. However, the proportion of sum of squares due to GCA was always much larger than SCA in each character. It may be concluded that additive genetic effects are more important than non-additive genetic effects in determining the inheritance of the observed characters.

In this genetic material, certain parental varieties, such as Hana, gave low performing F_1 hybrids. All F_1 hybrids which had this as a female or male parent were lower than all other F_1 hybrids. Therefore, these F_1 hybrids decreased the average of all F_1 hybrids.

Concerning heterosis in wheat anther culture, many investigators, for example Lazar et al. (1984b) obtained similar results in which they reported the presence of heterosis over the mid-parents (M.P).

The absence of heterosis from the better parent for most hybrids could be explained as a result of the advantage of general combining ability effects with respect to specific combining ability effects.

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CHANGES IN THE Cu-, Mo- AND CRUDE PROTEIN CONTENTS OF ALFALFA VARIETIES ORIGINATING FROM DIFFERENT AND IDENTICAL SOIL TYPES

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Changes in the copper-, molybdenum- and crude protein content of the first growth of alfalfa varieties originating from different and identical soil types, respectively, in the same stage of development were studied on the basis of 217 and 157 samples, respectively.

According to the results the Mo content of the alfalfa varied with the soil type, the largest quantity of it (3.82 mg/kg dry matter) combined with a low Cu content (7.5 mg/kg dry matter) was contained by alfalfa stands in marsh- and peat soils; in the crude protein content differences depending on the growing site could not be detected. The Mo-content of alfalfa grown in acid soils, though low (0.40 mg/kg dry matter), is 3-4-times the amount required for the animals.

Between the Mo- and crude protein content of alfalfa varieties grown in the same type of soil there is a positive correlation, while with the Cu content a significant negative correlation can be pointed out. Varieties incorporating more protein have a higher Mo content compared to those with a lower protein content, their copper content, on the other hand, decreases parallel with an increase in the crude protein.

Keywords: alfalfa, copper, molybdenum, crude protein

Introduction

The molybdenum is an element equally essential for humans and animals. De Renzo et al. (1953) and Richert and Westerfield (1953) identified the molybdenum as a component of the xanthine dehydrogenase enzyme. Its vital importance was determined by Payne (1977) for poultry, and by Anke et al. (1977) for ruminants. Earlier only the harmful effect of Mo was known (Ferguson et al., 1938) and, until now, most works have dealt primarily with the toxicity of Mo (Cunningham and Hogan, 1958; Henning, 1972; Anke and Risch, 1979; Zumkley, 1983; Underwood, 1977, etc.).

In crop production the legumes have a particularly high demand on Mo, since the root rhizobia need molybdenum for fixing the nitrogen of the air (Steinberg, 1937), but Mo deficiency may also occur in other crops (Bergmann, 1983), which can result in reduced yields, yellow leaves, deficient inflorescence, decreased protein content and increased proportion of amide within, and may involve increases in carbohydrate and ash content (Anke et al., 1960, 1963; Anke and Gruhn, 1962; Pais, 1980).

Of all plant species the legumes store the most Mo (Döll, 1976). The French bean e.g. contains more than 2.5 mg/kg dry matter Mo under normal conditions. Plant species rich in leaves, such as grasses, lettuce, and whole maize plant contain Mo between 0.3 and 0.7 mg/kg dry matter. The seeds of legumes contain above

5 mg/kg dry matter, while the grains of cereals are poor in Mo (0.13–0.24 mg/kg dry matter).

The uptake of Mo and Mn shows opposite trends (Tölgyesi, 1969). The Mo content of alfalfa grown in acid soil is much lower than 1 mg/kg dry matter, while in calcareous and alkali soils it may contain several times more Mo than that; of manganese, on the other hand, 60–70 mg/kg may even be in the dry matter of alfalfa at low pH values, but in loess soils only half that quantity (28–32 mg/kg) can be found in it.

Materials and methods

The samples were collected from the first growths of various alfalfa varieties grown in different soil types (n = 217) and in the same type of soil (n = 157), respectively, in the same phase of development, at the time of budding. The preparation of the samples was carried out according to Régius and Szentmihályi (1975): drying at 60 °C, then at 105 °C to steady weight, reduction to ashes at 450 °C. The crude nutritive elements were determined by Weendei analysis, the minerals with atom absorption spectrophotometer, and the molybdenum colorimentrically with dithiol. The data are given in terms of dry matter. The soil types are classified after Stefanovits (1963).

Results

The change in the mineral composition of plant parts—stalk, leaf, inflorescence—is important in regard to feeding, because at the time of harvesting the proportions depending on the stage of development and on possible losses vary. The foliage is the richest plant part in mineral content, so its preservation on hay making is important.

Table 1 shows the crude protein-, Cu- and Mo composition of alfalfas grown in different soils.

According to the data alfalfas grown in marsh- and peat soils are very rich in Mo (3.82 mg/kg dry matter), which is combined with a relatively low Cu content (7.5 mg/kg dry matter). In clayey- and Trias alluvial soils the lucernes have a Mo content somewhat higher than what those grown in loess have; the lowest Mo content was found in alfalfas grown in acid sandsoils. According to literary data (Anke et al., 1984, 1986), the Mo content of the vegetation is higher in alkali than in marsh soils, as shown by the relative values in Table 2. If we disregard the soil classification of Stefanovits and take instead the geological origin of soils in the sampling places for our basis, then we obtain the values contained in Table 3. Alfalfa in alkali soils contains 31% less Mo than in marsh- and peat soil, and the lowest value was given by the alfalfa stand of the acid sand rather than by that grown in andesite soils. This difference is supposedly caused by the fact that the values published by Anke et al. (1984, 1986) refer not only to alfalfa but to so-called indicator plants (alfalfa, red clover, wheat, rye), and in the "relative values" the specifically different Mo contents of the plant species examined also have a part. The trend of the crude protein content shows no Mo- or Cu dependent differences. The crude protein content of alfalfas at the beginning of budding is in every case high (22–26% of dry matter) and characteristic of the young alfalfa plant. Anke et

Table 1

Crude protein-, Cu- and Mo contents of alfalfa grown in different soils

	n	Crude protein %			Cu mg/kg			Mo mg/kg		
		$\bar{x}$	±s	CV %	$\bar{x}$	±s	CV %	$\bar{x}$	±s	CV %
Loess soils										
Compact prairie soil	45	25.90	4.12	14.27	15.16	6.38	42.08	1.31	0.66	50.38
Sandy Prairie soil	15	21.92	4.10	18.76	8.30	4.44	50.45	0.84	0.41	48.81
Compact meadow soil	19	25.15	3.28	11.68	11.75	3.41	29.02	0.94	0.54	57.45
Forest soils										
Chernozem brown forest soil	15	23.23	3.08	11.99	11.89	4.20	35.32	0.63	0.40	63.49
Brown forest soil with clay infiltration	22	24.00	2.96	11.14	12.56	4.06	32.32	0.82	0.51	62.20
Alluvial soils										
Meadow alluvium	26	26.00	2.87	9.89	16.40	1.82	11.10	2.54	0.61	24.02
Clayey alluvium	12	24.14	3.42	13.33	10.50	1.60	15.24	1.38	0.21	15.22
Tisza alluvium	9	24.92	2.74	10.99	16.75	2.50	14.93	1.28	0.90	70.31
Sandsoils										
Calcareous	7	25.62	8.67	30.58	9.40	2.30	24.47	1.00	0.50	50.00
Acid	9	22.00	3.62	14.80	9.20	1.79	19.46	0.40	0.12	30.00
Alkali soils	21	25.00	3.18	12.50	14.75	2.43	16.47	2.63	0.41	65.08
Marsh soil	17	25.72	4.06	14.83	7.44	2.01	27.02	3.82	1.56	40.84

Table 2

Soil specific Mo contents of indicator plants as a percentage of plants grown in soils richest in molybdenum (Anke et al., 1984)

Soils	
Alkali soils	100
Marsh, peat soils	80
Alluvial soils	62
Calcareous sandsoils	54
Loess soils	45
Acid sandsoils	33
Andesite soils	21

al. (1960, 1962, 1963), Graupe et al. (1960) were able to increase both the protein content and the yield of alfalfa by Mo nutrition in places where the soil was Mo deficient or the available quantity of Mo did not cover the requirements of the plants. However, in alfalfas grown in such soils 0.02–0.06 mg/kg Mo was found in the dry matter which falls far behind the Mo content of the acid sandsoils.

Table 3

Mo contents of alfalfa samples as a function of the geological origin of the soil (mg/kg dry matter)

Soils		
Marsh, peat soil	3.82	100%
Alkali soil	2.63	69%
Alluvial soils	1.73	45%
Calcareous sandsoil	1.00	26%
Loess soil	1.00	26%
Andesite soils	0.72	19%
Acid sandsoils	0.40	11%

The crude protein-, Cu- and Mo contents of various alfalfa varieties grown in the same (sandy prairie) soil type are contained in Table 4 in order of increasing crude protein content. The values seen in the table reflect the interaction of Mo and Cu. The higher protein content is combined with more Mo, and the higher Mo content with a smaller quantity of Cu.

Table 4

Crude protein-, Cu- and Mo contents of various alfalfa varieties grown in the same type of soil, as grouped by crude protein content

		Crude protein %	Cu mg/kg	Mo mg/kg
—18 % crude protein content	$\bar{x}$	17.6	12.3	1.3
	±s	0.47	3.01	0.27
	CV %	2.7	24.5	20.8
18—19 % crude protein content	$\bar{x}$	18.7	10.5	1.7
	±s	0.25	3.88	0.29
	CV %	1.3	37.0	17.1
19—20 % crude protein content	$\bar{x}$	19.5	10.1	1.8
	±s	0.27	2.89	0.43
	CV %	1.4	28.6	23.9
20—21 % crude protein content	$\bar{x}$	20.5	9.7	2.1
	±s	0.66	2.81	0.59
	CV %	3.2	29.0	28.1
21—22 % crude protein content	$\bar{x}$	21.4	9.2	2.4
	±s	0.23	1.53	0.54
	CV %	1.1	16.6	22.5
22— % crude protein content	$\bar{x}$	22.4	8.6	3.1
	±s	0.31	1.4	0.51
	CV %	1.4	16.3	16.5

Alfalfa varieties able to take up more Mo from the soil supposedly are able to fix more N from the air with their root rhizobia, whereby their crude protein content increases. According to Anke et al. (1963) Mo-deficient alfalfa contains less crude protein than that grown in plots supplied with Mo, but Mo nutrition increased the protein content of alfalfa even in stands not deficient in Mo.

Figure 1 shows the correlation of crude protein and Mo. The extent of correlation is between $r = +0.24$ ($P > 0.5$) and $r = +0.78$ ($P > 0.01$), it is an average of $r = +0.57$, $P > 0.1$. This confirms the statement of Anke and Gruhn (1962), Anke et al. (1960, 1963), Falke (1984), namely that the protein incorporation is a process

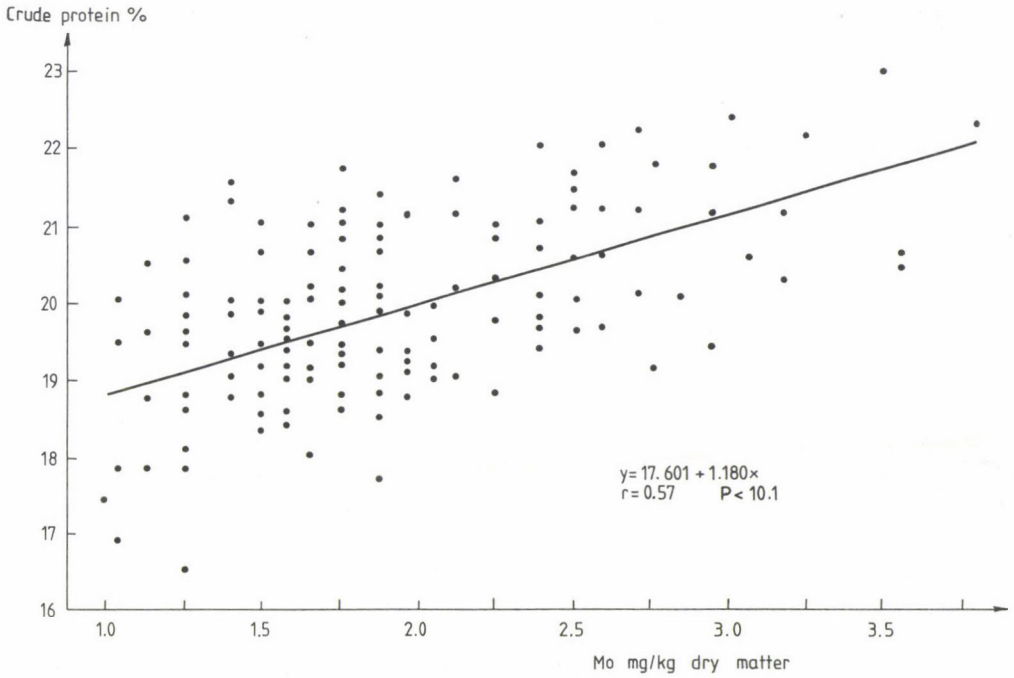

Fig. 1. Correlation between the crude protein- and Mo content of various alfalfa varieties (n = 157) grown on the same area (ny.feh. = crude protein)

linked with Mo. In the case of a Mo supply suited to the requirements those alfalfa varieties as able to produce more protein take up more Mo, too, and store it not only in the root rhizobia but also in the vegetative plant parts.

Figure 2 represents the negative ($r = -0.25$, $P < 0.5$) correlation between the crude protein and the Cu content which, in fact, suggests the antagonism of molybdenum and copper (Hennig, 1972; Pais, 1980; Underwood, 1977).

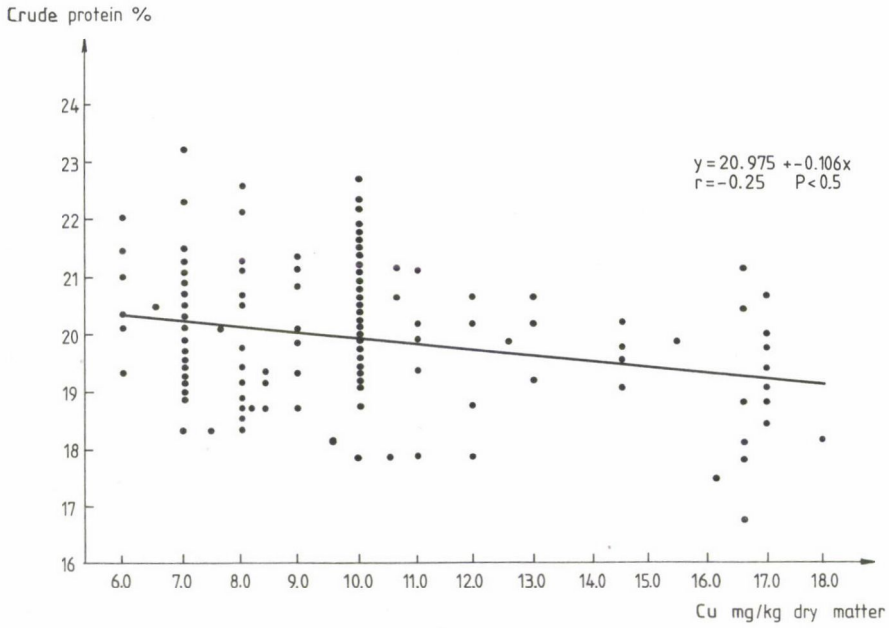

Fig. 2. Correlation between the crude protein- and Cu content of various alfalfa varieties (n = 157) grown on the same area (ny.feh. = crude protein)

Discussion

The geological origin of the soil influences the molybdenum content of plants. However, the quantity of available Mo greatly depends on the pH of soil. In our experiments the alfalfas grown in acid sandsoils contained the smallest quantities of Mo, 0.4 mg/kg, at CV 30%. Rousev (1986) observed a 12% increase in the crude protein content of alfalfa hay when fertilizing the alfalfa stand of a soil poor in molybdenum with Mo; the result was 0.74 mg/kg compared to 0.18 mg/kg in the control. Anke et al. (1963) found that even in case the amount of available molybdenum covered the requirement of the plants, molybdenum nutrition somewhat increased the protein content. In our present study the crude protein content of alfalfas grown in different soil types showed a much lower fluctuation compared to Mo, which suggests that the lowest available Mo content of the soils proved sufficient for fixing the necessary nitrogen and converting nitrates, respectively.

Alfalfas grown in different soil types showed a nearly 90% variation of Mo content, but between the extreme values of their crude protein content the difference did not reach 20%.

Between the crude protein and molybdenum content of various alfalfa varieties grown in the same type of soil significant correlation was found, which unambiguously confirms the role played by Mo in the nitrogen metabolism (Grün et al., 1989).

From a point of view of animal feeding Mo plays an important role in the ruminal digestion, taking part in the microbiological decomposition of nitrates. Therefore, the Mo requirements of ruminants are somewhat higher than those of monogastric animals. This means that while the Mo requirement of ruminants slightly exceeds 100 μg/kg dry matter, that of pigs e.g. does not quite reach this quantity. As mentioned in the introduction, the grain crops belong to the feedstuffs poor in Mo, which, however does not mean that with an extremely large proportion of grains, Mo deficiency must be reckoned with, since even the grain contains in every case 100 μg/kg dry matter (Anke et al., 1984).

The animal species give different responses to an oversupply of Mo, cattle being the most sensitive, followed by sheep, horses and swine, while goats shows the least sensitivity. However, toxic symptoms even in cattle only appear in the case of a Mo content exceeding 10 mg/kg fodder dry matter, and this amount was not even contained in alfalfas grown in marsh soil, the type that is richest in Mo. Yet it is true that the vegetation of marsh soils carries little copper beside the higher Mo content, which may lead to Cu deficiency. The low copper content of the feed may cause primary, the high Mo content secondary deficiency, which greatly reduces the Cu content of the different organs and results in all kinds of deleterious effects. According to Tölgyesi (1969), the deleterious effect of Mo may appear when the ratio of copper to molybdenum is less than 5 : 1, and this ratio is not reached by the alfalfa of marsh soils, since beside 7.5 mg/kg Cu we found 3.82 mg/kg Mo, so the Cu : Mo ratio was 2 : 1. In the practice of feeding feed rations with high Mo- and low Cu contents need Cu supplementing, particularly in the grazing system of keeping, when through a complementary feeding this narrow ratio is not corrected.

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MARKET REFORM IN TRANSITION ECONOMIES

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The collapse of command systems poses an immense challenge for conversion to more market-oriented economic systems. In the process, the architecture of market structure, processes, and environments must be built on the historical foundation and context of the society as it engages in the transformation struggle. The measurement and analyses of evolving market structures, processes, and behaviors are necessary to provide strategic guidance for the reform process. This paper presents a strategic guidance system for the allocation of scarce public and private resources, to maximize the development and economic well-being of society. The approach and methods are rooted in the discipline of macromarketing and, if implemented, can have a significant effect on critical decisions in the transition process.

Keywords: channel, channel mapping, demand behaviour, demand side, distribution systems, driving force, investments, needs of customers, policy orientation, privatization, strategic guidance system, supply side, transition economy, vertical coordination

Introduction

Francis Fukuyama (1990) has declared the end of history, with the ultimate economic conquest of markets over command systems. The crumbling of the Iron Curtain and the decline of communism initiated an unexpected and overwhelming politico-economic chain reaction. Almost overnight, in a historical sense, whole populations and their politicians turned to market mechanisms to solve development problems and deliver a quality of material life comparable to that in the West. The effects of these bold moves have placed new emphasis on market development, and new pressures on command structures to give way to market mechanisms and/or to provide an environment which enables those mechanisms to grow. Dixon (1991) describes the fundamental transition as "a change from a system characterized by the 'Dictatorship of Needs' to one characterized by the ability of consumers to express their demands".

The transition process represents a drive to achieve societal goals through the infusion of market concepts in societies with established command-driven political and economic institutions, with many generations of command-oriented socialization, and with weak economic resources in terms of capital and technology. The transition process itself, which is unparalleled in history, is one of great risk and uncertainty. Even so, there exist many variants of market-driven economic systems in the world, and there is no lack of consultants who would advise on the transition

process from their experiences and perspectives. What seems to be missing is a method, rooted in the existing realities of current transition economies, of approaching key resource allocations and process definition decisions. In other words, a blueprint is needed which operationally specifies the steps and activities necessary for the provision of relevant guiding information. In short, what is missing is a strategic guidance system for market reform. The purpose of this paper is to suggest a strategic guidance system that can be applied to sectors or industries in order to allow effective allocation of scarce development resources. This approach draws its focus from macromarketing; that is, from a perspective which combines societal long-run objectives, a method of measuring marketing behaviour and structure, and the knowledge of marketing systems.

The food system should be singled out for special attention in the Hungarian economy for four reasons. First, the Hungarian food and agricultural industries have shown significant progress towards a competitive market economy, relative to other Central and Eastern European (CEE) countries. In order to enable competition on the world market, however, further improvements in both the vertical and horizontal market coordination are needed. This challenge is crucial for the future development of the Hungarian food and agricultural industries.

Second, the agrofood sector has been the subject of some of the greatest reforms with a major program of privatization and rationalization of the use of productive capacity. However, probably more than any other market within the economy, the food sector is a system which ranges from agriculture at one end to the consumer at the other, with a multitude of intermediate stages. For the sector to operate effectively within a market economy, an integrated system has to be developed whereby consumer demand effectively flows back ultimately to influence primary production.

Third, the success of the food sector is also vital to the political, social and economic stability of the country as a whole. With the opening up of the country, consumers have begun to expect adequate supplies of a wide range of basic food supplies, as well as innovative products such as are available in the West.

Finally, the impact of the food system on the economy is important because of its share of export earnings (30%) and also because consumers spend about 40% of their disposable income on food.

Conditions for market operation

In order for a market system to operate, a certain minimal market infrastructure and a market institution system must be present. The more complete the infrastructure, the more likely the market is to operate effectively and efficiently. The key elements of this infrastructure are noted below.

Property rights. The fundamental right inherent in a market system is the right to ownership of what is to be bought or sold (Harris and Carman, 1983; Dixon, 1991). This implies a legal system which protects the freedom of choice in the market, the right to property, the right to contract, and the right to have contracts

honored and enforced. These right are made possible by the laws established by the Parliament in the last four years.

Information systems. Markets cannot work without the exchange of information. For any development beyond face-to-face trade among neighbour, a communications infrastructure must exist. This includes not only telephone linkages but also information from a vast variety of sources. These may be as simple as the direct observation of prices and availability in a physical market, or as complex as computerized data bases. The sources may be private providers or government agencies. The efficiency and effectiveness of market mechanisms are also significantly affected by the veracity of the information. Here again, a legal and regulatory infrastructure is necessary to insure the integrity of information, whether it relates to market operations or to products and services.

Physical infrastructure. Effectively meeting market demand requires a vast array of physical and transactional functions. Obvious ones are transportation, storage, bulk breaking, financing, security, and inventory control functions. Less obvious are the channels of distribution functions that create assortments of goods and services, such that prospects can have access to them when and where desired, and in a form and on the terms desired.

Societal imperatives. Every marketing action has societal effects, both positive and negative. Therefore, marketplace behavior needs to be molded to insure that the interests of the larger society are achieved. For example, the continuity of development over time; the effects on the physical environment, culture, and social development; the safety of products (especially food and pharmaceuticals); and many other issues need to be addressed by the rules that are set up to guide the market system. Since the rules themselves often have unintended consequences and failures (Carman and Harris, 1986), the rule-making process must be guided by careful analyses of main and secondary effects as they occur in a specific market environment or situation.

Many additional elements of marketing infrastructure enhance performance. The most important relate to the access to capital and loans (financial markets), bankruptcy laws and procedures, insurance programs, stable convertible currencies, and last but not least, adequate training facilities.

Such an infrastructure has to be established at the societal level. Consistency over time, and fairness of application towards individuals and organizations are no less important than the substantive quality of the infrastructure in fostering marketing behavior which enchances the well-being of society. The strategic guidance system proposed here is designed to provide analyses that can improve not only the marketing infrastructure established at the national level, but also the effectiveness of markets and marketing within specific industries and channels of distribution. The question is how to deploy scarce development resources (time, legislation, and capital) to enhance the formation, rationalization, or operation of markets. The guidance system will certainly lead to ways of improving the infrastructure, but it also focuses on market processes, on the behaviour of market participants, and on those barriers which still have to be removed to facilitate the transformation process.

Preconceptions about markets in transition

Most of the focus in transition economies is at the macroeconomic level. The underlying assumption is that, if economic activity can be privatized, markets will drive the system to efficiency and economic development (Slater et al., 1969a). Given the experiences in many developing regions, this is a flawed assumption. As Collins and Holton have noted (1964), "rarely. . . is it recognized that industrial and agricultural sectors in turn are dependent on the development of a distributive sector to bridge the gap between producer and ultimate consumer".

There is a strong conviction by many that privatization and rapid Western investment programs are the only missing elements in the transition process, and the lack of capital and competitive technology are the only limiting factors. The inflow of investment into the country is not likely to contribute to the transition process unless the whole vertical channel of the industries involved (a holistic approach) is considered.

In addition, there is a lack of operational marketing knowledge, not in terms of what a market can do in principle, but of how to put it in place. The promise of market systems is well known, but the nuts and bolts are not well understood. In economies where generations have experienced only shortages, and where goods and services of only very poor quality were offered, the notion of market need as a driving force is a *non sequitur*. The means for understanding a need and translating it into customer satisfaction in a competitive environment are almost wholly lacking. Results from 75 interviews (June 1991) with academic, business, and government leaders in Poland, Hungary and Czechoslovakia clearly support this conclusion. The plight of the transition in terms of insufficient capital, the need for privatization, the lack of world competitiveness, the loss of domestic and former Soviet bloc demand for domestic products, the absence of marketing expertise, and the deficiency of exporting expertise were well understood by those interviewed. Pervasive in their thinking was the perception that the only means of survival were through joint ventures and strategic alliances with, or absorption by, Western firms. Through such arrangements, it was hoped that access to Western markets and technologies (including marketing, management, production, and quality control) could be acquired. An approach to the development and reform of specific markets at the operational level was not in evidence.

Development in the context of existing institutions and processes

The question is not what a market system can do in principle, but what specific actions need to be taken to enable it to fulfill its promise. Yet the question to be seriously addressed in the transition process is what actions to take, and how and when to implement them. These actions necessarily will occur in the context of current practices and institutions. In other words, the reform process must be applied to an existing marketing structure with its institutional and behavioral attributes. This notion of market rationalization in the context of existing struc-

ture, process, and heritage, is a key element of a strategically sound guidance system for the development of resource allocations.

It is true that many institutions and practices eventually will be replaced, but the existing values, culture, and infrastructure will have a pervasive influence on the transformation process. To ignore this reality and assume that the new can be created in a vacuum, free of the past, is ludicrous. For example, who will manage the economic organizations? Where will many of these organizations originate? The process of privatization simply converts ownership; the entities remain. Certainly, many new businesses will be started, but many of the entrepreneurs will emerge from the former black market. In terms of larger enterprises, who are the new managers? Any planning process which does not start with a careful knowledge of current institutions and their prospective leadership is seriously flawed. The intent is not to perpetuate existing economic structures and processes, but to use what exists as a springboard for change that will have a realistic prospect for survival against the forces of history and the change process itself. The context is essential in redesigning the architecture of the economic system.

From a study of the literature of marketing and development conducted by Joy and Ross (1989), several conclusions emerged.

"Marketing strategies are most likely to succeed if they take into account the factors that led to a society's development. Marketing programs... must take into account the strengths and weaknesses of local and regional institutions. Marketing programs that begin with local constructions of social reality through careful cultural analysis are more likely to succeed. New technologies, ideas, and values will be accepted only if they meet real needs of people".

Considerations about the method

The attractiveness of the market system concept is predicated on three key notions.

— First, customers (individuals or organizations, foreign or domestic) provide the guidance for the production of goods and services.
— Second, self-interest provides a strong motivation for behaviour.
— Third, competitive systems can help to guide market behaviour toward societal well-being.

Theoretically, producers and providers in a market system will try to serve their self-interest by better satisfying the needs of customers than do their competitors. Customer needs and demands provide the dynamic force. Competitors will continually seek ways to supply greater satisfaction, and those who do less well will have to redouble their efforts to win a profitable volume of sales.

This idealistic vision of market behaviour and its dynamic benefit to society can occur only within a context of those institutions and controls that prevent a wide range of behaviours which detract from the welfare of the system (Harris and Carman, 1984). Thus, guidance systems to prevent abuses are needed as part of the structure and process of a market system.

If the customer demand drives a market system, then there is a compelling argument that any guidance system must start with an analysis of consumer needs and behaviours, and work backward through the channel of distribution (Dahringer, 1983). Even though Hungary is unique in its current institutions and consumer attitudes, the principle of market-driven development remains fundamental to transition.

Furthermore, where the market channel is not effective or efficient, it is important to start the reform process by clarifying the ability of customer need to direct the allocation of resources through the channel to the producers. Market rationalization should begin with the final market and proceed toward the sources that supply it, rather than applying a focus that moves forward from production. In competitive situations, channel members and producers tend to look toward their markets to catch the critical signals for what and how much to produce. A lack of clarity regarding market demand, dynamics, and needs caused by inefficiencies and distortions of the channel in front of them, on the demand side, results in increased risks and uncertainties as to the payoff of alternative management strategies. These risks and uncertainties are likely to retard investment, innovation, and aggressiveness.

This process can be visualized from the vantage point of channel members as each looks toward the market. If inefficiency and disorganization on their demand side discourage their own rationalization, then the logical place to start the reform process is with the institutions that serve final demand (Cundiff and Hilger, 1980). This may range from retailers or sellers to industrial buyers. For example, in the case of consumer markets, rationalized retail performance would then provide better markets for the next channel members in line, and so forth, along the channel to the point of production. This is not to say that the supply side of any member of the channel is not problematic in transition economies. The chronic shortages in Central Europe and the former Soviet Republics attest to these problems. Where private initiative motivates organizational behaviour, however, there is less incentive to resolve the supply side problems if the demand side risks and uncertainties are high. Rationalizing the channel by moving backward from final demand provides the incentive to rationalize the supply side problems of each channel member, including the producers. The reverse channel rationalization process encourages vertical cooperation, coordination, and integration, which in turn promote efficiency and effectiveness of markets (Slater, 1968, 1976, 1977).

Measurement of marketing institutions and processes

What exists in terms of market practices, institutions, and channels of distribution (the linkage of practices and institutions) must be identified, mapped, and analyzed. The purpose is to monitor ongoing change and measure the opportunities for intervention on both micro and macro levels for specific industries and channels. This process of channel mapping was developed by Charles Slater and his colleagues in a series of pioneering studies in Latin America (Slater, 1968; Slater et al., 1969a,

1969b; Riley, 1967, 1970; Harrison et al., 1974). Channel mapping examines several components of the distribution system.

Demand behaviour. As noted above, analyses should start with the demand and the behaviour of customers. Three classifications of customers need to be examined: domestic consumers, foreign export markets, and industrial/institutional buyers. In all three cases, the analysis needs to identify sources of income, buyer behaviours, location and type of sellers, needs and attitudes toward the products/services and the conditions of the selling process, attitudes toward change, and the competitive dynamics of the marketplace (Slater et al., 1979). The key questions are: Who are the buyers? What do they buy? From whom do they buy? Under what conditions are the purchases made? Where are the transactions conducted (Kaynak and Cavusgil, 1982)?

Trade channel characteristics. The trade channel analysis traces the links between final or industrial buyers and producers. It may include retailers, wholesalers, distributors, exporters, sales agents, manufacturer's representatives, and a multitude of different combinations. The objective is to understand the market structure and marketing systems, both in operation and as they change over time. Key elements involve identifying the participants, power and leadership relationship, efficiencies and inefficiencies, value added, barriers and opportunities for rationalization, attitudes toward change and innovation, motivations, capabilities and expertise (Slater et al., 1979). Any ease of entry along the channel and opportunities for vertical coordination and cooperation must also be investigated. The key questions relate to where change agents and change blockers exist, where barriers and obstacles exist, where intervention could rationalize the channel to make it more effective and efficient in the delivery of customer satisfaction, and how market incentives can foster a cycle of positive economic development.

Exporter analysis. This category of channel member deserves special mention to highlight the importance of export markets and the channels that cultivate and serve them. The same issues and questions should be raised as with other channel components, although the international dimension is a unique feature. In Central Europe, where export marketing traditionally has been handled by government trading organizations, these channels often lack sophistication and development, if they exist at all.

Producer/processor linkages. Value-added manufacturing and processing take their direction from the market through the channel of distribution and through other primary and secondary data sources. Because production is so critical to the performance of the system, it is the focus of this analysis. The same questions are asked as was the case with the trade channels. It may be important to trace the channel beyond the producer back to ultimate sources of supply, but this will depend on the industry and its supply characteristics. For example, processed agricultural products generally require this thorough an analysis.

Slater et al. (1979) conclude that "this channel mapping methodology is positive, descriptive research with a policy orientation". It is designed to provide an integrated analysis of the marketing system for a product/service or industry category, such that micro- and macro intervention strategies can enhance value-

added benefits to the development process. In its simplest form, this channel mapping takes the form of a flow diagram of the marketing institutional linkages. In its more complex form, channel mapping identifies the change agents and the barriers or blockages to more efficient market operations. Participant attitudes and perceptions are measured as well as the physical flow of goods and services (Nason and White, 1981).

A strategic guidance system

The purpose of the channel mapping outlined above is to isolate specific strategies that can improve the performance of the market system for the benefit of the larger society. What intervention strategies have the greatest effect, given scarce resources? Who should be trained? What kind of training is needed? Where should credit be made available? Where is insurance critical to reduce market risks? The underlying principle of channel mapping is that such an analysis enables scarce development resources to be targeted more efficiently and effectively toward market development needs. Not only are more efficient markets needed, but also ones which will contribute to sustained development in an environmentally friendly manner. Channel mapping results in providing information about the market barriers to remove, and to foster in institutional system so as to help the coordination between market participants. This is true in both the domestic and international channel context.

Conclusion

The transition of command systems represents an immense challenge and a significant risk to macromarketing as a discipline. The challenge is to identify and measure marketing system dynamics in order to guide development efforts. To the degree that macromarketers can provide the analytical techniques and the appropriate marketing technologies needed to achieve the transition, they will gain the recognition commensurate with their contribution. Normative macromarketing can provide guidance on such matters as property rights, contractual arrangements and enforcement, information provision and integrity, marketing institutions and linkages, marketing systems and functions, and marketing infrastructure (transportation, communication, wholesale channels, and so forth), as it is the rationalization of the environment of marketing that enables a sustainable and environmentally friendly economic development. The guidance of the environment, within which marketing action occurs, is the domain of macromarketing.

The risk to the discipline is that the market system will not materialize in time to prevent economic disillusionment and the resultant political paralysis or reversals. The study of market failure is as much within the domain of macromarketing as is the study of positive effects, but much of the potential positive enthusiasm for the discipline will be negated. The appropriate agenda for development will be harder to sell and implement, even though failures may be inevitable, due to the magnitude of the task in each market. The consequences to society of market failure

depend on its type and degree. Harris and Carman (1983) present a typology of market failures useful for the macromarketing analysis suggested here. They go on to analyze regulatory responses to market failures (Harris and Carman, 1984) and regulatory failures (Carman and Harris, 1986), all of which are germane to macromarketing.

Market principles place marketing in the economic limelight. But the performance of marketers in delivering a standard of living depends on the enabling of a macromarketing environment, created through enlightened policy, guided by macromarketing measurement and analysis. Thus, the transition of command economies represents a major opportunity for elevating the value of macromarketing.

The logic in a strategic guidance system for market reform in transition economies can be summarized as follows.

1. The assumption that marketing, marketing institutions, and marketing behaviours will develop naturally and adequately with the demise of command institutions is flawed.
2. The real vacuum that remains to be filled in the transition process relates to the operational measures needed to enhance the effectiveness and efficiency of the marketplace.
3. These operational measures need to take place in the context of marketing structures, institutions, and behaviours as they exist and as they change over time.
4. These marketing structures, institutions, and behaviours must be measured over time to obtain guidance for targeted reform efforts.
5. Because the macromarketing measurement and analysis task is so large, it is proposed that it should be the major research agenda for home country academic institutions and faculty.
6. Coordination and facilitation of research management will be needed to insure quality, consistency, and cross cultural comparability of results. It is likely that this would have to come through the auspices of external donor agencies.
7. The outcome of such a macromarketing thrust would be threefold. First, it would contribute to the development process itself. Second, it would empower national academic institutions and faculty with the expertise which comes from the research process, providing them with intimate knowledge of markets and industries, as well as cross-cultural insights. Third, it would yield knowledge about transition economies, generalizable strategies to guide reform, and economic development.

Preliminary investigations indicate that no other projects are pursuing such ground level monitoring and guidance. Somehow, policies of intervention are being divined at the aggregate level. Certainly, macro policies are important in the broad restructuring of the competitive environment, but many such strategies will be wasteful and irrelevant, if the real needs of those in the forefront of change are not taken into account.

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Animal physiology and biochemistry

COMPARATIVE STUDY OF THE NUTRITIVE VALUE OF EXTRACTED MEALS PREPARED FROM VARIOUS HUNGARIAN AND FOREIGN SOYBEAN VARIETIES

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The authors carried out chemical and biological comparative examinations of 18 foreign- and 12 Hungarian bred soybean varieties originating from Hungarian comparative variety trials. The study included the determination of the chemical composition (dry matter-, crude protein-, crude fat-, crude fibre- and ash content) of the soybean. The soybean samples were extracted and exposed to wet heat treatment and, after measuring the trypsin inhibitor activity of the extracted soybean meal, the amino acid composition of the proteins, further, in N-metabolism experiment with rats the biological value, digestibility, net- and productive conversion of protein were studied.

On the basis of the results of examinations, between the crude protein- and crude fat content of the soybean a significant negative- ($r = -0.764$ and $r = -0.512$, respectively) while between the crude protein- and lysine content a significant positive ($r = 0.700$ and $r = 0.646$, respectively), medium correlation was determined. Neither the chemical analyses, nor the protein utilization parameters obtained in animal experiments, showed any significant difference between the extracted and heat treated foreign and Hungarian soybean varieties.

Keywords: soybean, amino acid composition, N-balance, biological value and true digestibility of protein

Introduction

During World War II the cultivation of soybean was considerable in Hungary. Then, after a sharp decline, interest in it began to grow in the 1970s (Bódis and Kralovánszky, 1988).

In Hungary soybean is taken into account as a source of protein beside the cereal energy carriers in animal feeding, primarily in extracted form. However, we cannot be self-suppliers of soybean, because the geographic conditions of Hungary limit the increase in the production area of soybean, which means that only a proportion of the demand of soybean can be fulfilled by domestic production, and imports cover the larger part of the soybean requirement. The size of the soybean yield is determined by the interactions of environmental factors, genetic characteristics of the varieties and production technologies. It is up to the breeder to coordinate these factors. On choosing the variety, the target of production has to be taken into consideration as well as the ecological and economic conditions. If the purpose of production is to use the soybean for human nutrition or for feeding animals, then—according to Bódis (1983)—it must be regarded as a protein crop and its value is best considered as such.

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The trypsin inhibitor present in the soybean checks the utilization of protein in the animal organism, which can be lessened by a suitable treatment (Hansen et al., 1987; Chang et al., 1987). For the conversion of the soybean varieties, inactivation of the trypsin inhibitor by heat is the most important (Hegedüs et al., 1981).

Mieth et al. (1988) studied the effect of genetic characteristics and environmental factors on the chemical composition of soybean. They found a negative correlation between the crude protein content and the crude fat-, N-free extractable matter content and the urease activity, but a positive one between the increasing crude protein content and the lysine-, cystine- and methionine content. According to the authors mentioned above, the variety specific differences of soybean appear in the chemical composition to a lesser extent than do the environmental (soil, climate, etc.) effects. They found that the climatic factors had a significant effect on the protein- and fat content of the soybean as well as on its amino acid- and fatty acid composition.

All this data make it reasonable to examine the soybean as a protein carrier by chemical and biological methods.

In our experiments, 12 Hungarian and 18 foreign-bred soybean varieties grown in 1988 at the same place were compared from the standpoint of animal feeding, with special regard to the amino acid composition of the soya protein and its *in vivo* utilization in monogastric animals.

Materials and methods

The Seed Production and Marketing Enterprise placed at our disposal the 30 soybean samples originating from variety trials in Hungary; they were grown at the experiment station of Türje; forecrop: soybean; soil: prairie loam, chernozem: sowing time: 3 May; fertilizer: 100 kg N, 110 kg P, 110 kg K; annual precipitation: 403 mm.

According to the standard MSZ-6830, we determined the nutrient content of the soybean samples (dry matter, crude protein, crude fat, crude fibre, ash, N-free extracted matter).

Having determined the total nutrient content of the soybean seed we extracted the samples with ethylic ether, then exposed them to heat treatment at 110 °C for 20 minutes, in an aqueous medium. The heat treatment was needed for inactivating the trypsin inhibitor of soybean. The trypsin inhibitor activity was investigated using the TI-test paper of the Scientific Production Association of Protein Technology.

The amino acid composition of the extracted and heat-treated soybean meals was determined by means of an Aminochrom-II type amino acid analyser.

The biological value, true digestibility and net- and productive utilization of the extracted and heat treated soybean proteins were established on the basis of N-metabolism test performed with rats (Szelényi, 1969). For the experiment, semi-synthetic diets were composed in which the only source of protein was represented by soybeans. In the diets the fat demand of the rats was satisfied by sunflower oil, while their carbohydrate demand was satisfied by sugar and potato starch. In addition, the rats were supplied with an appropriate mixture of vitamin and mineral.

After 4 days of preliminary feeding the N-metabolism experiment was carried out in a 5-day collection period with rats (5 animals per group) kept separately in metabolic cages.

Results

Chemical analyses

The chemical composition of the soybean varieties in terms of 86% dry matter content is shown in Table 1. Besides the designation of the variety, the country of origin and the time of maturing are also indicated.

The crude protein content of the foreign varieties is 32.2±3.6%, and that of the Hungarian ones 31.4±3.9%: the crude fat contents in the same order are 19.3±1.6% and 19.5±1.7%.

Table 1

Chemical composition of different soybean varieties, %
(in 86% dry matter content)

Variety			crude protein	crude fat	crude fibre	ash	N-free extr. matter
designation	place of origin	time of maturing	content				
S 1346	USA	I	29.0	19.0	5.3	4.5	28.2
Dawson	USA	0	32.4	20.4	5.1	4.8	23.3
Cx-117	USA	I	32.8	19.5	4.4	4.6	24.5
Cx-134	USA	I	34.8	18.4	4.5	5.0	23.2
Ewans	USA	0—I	27.4	21.3	5.9	5.1	26.3
Gadír	USA	I	24.8	22.3	4.0	4.9	29.9
B-070	USA	0	30.6	16.6	6.0	5.2	27.7
CM-048	USA	I—II	31.0	20.6	8.4	5.7	20.2
S-1460	USA	I—II	38.1	17.5	7.7	5.2	17.5
CM-137	USA	I—II	28.5	20.8	7.2	5.0	24.5
10305-01	Borza USA	I—II	30.3	19.2	4.3	5.6	26.6
Bora	Germany	II	38.2	18.5	5.7	4.4	19.2
Bonanza	Germany	0	35.3	17.6	3.6	4.7	24.7
Chandor	France	0	31.9	17.9	4.3	5.1	26.8
Apache	Canada	00	30.0	20.1	6.0	5.3	24.6
KG-60	Canada	0	33.8	17.6	5.3	5.0	24.3
KG-30	Canada	0	35.9	21.3	7.5	4.6	16.6
NS-16	Yugoslavia	I	35.3	18.3	6.1	4.6	21.7
$\bar{x}$			32.2±3.6	19.3±1.6	5.6±1.3	4.9±0.3	23.9±3.6
ISz-15	Hungary (Iregszemcse)	00	28.3	19.4	6.9	5.4	25.9
ISz-16	Hungary (Iregszemcse)	00	33.5	18.0	7.9	5.6	21.0
Gate M-511	Hungary	00	34.2	16.6	5.2	4.5	25.5
Éva	Hungary	0	33.3	18.8	4.9	4.7	24.3
Ákos	Hungary	0	39.4	16.7	4.3	4.1	21.3
Bolero	Hungary	0	36.4	18.8	5.5	4.0	21.1
BS-44	Hungary (Boly)	0	28.3	21.9	5.6	5.3	24.9
BS-45	Hungary (Boly)	0—I	26.8	19.7	6.0	5.7	27.8
BS-31	Hungary (Boly)	I	28.8	21.5	6.6	5.6	23.5
BS-56	Hungary (Boly)	I	27.3	20.4	5.3	4.8	28.2
BS-38	Hungary (Boly)	00	30.2	21.4	7.9	6.0	20.5
BS-247	Hungary (Boly)	0	30.0	20.6	8.0	5.3	22.1
$\bar{x}$			31.4±3.9	19.5±1.7	6.1±1.2	5.0±0.6	23.8±2.6

Out of the 12 Hungarian soybeans, the variety Ákos has the highest crude protein content (39.4%) followed by Bolero (36.4%); in the other varieties the crude protein values ranged between 26.8 and 34.2%.

In general, it can be said that varieties with relatively low protein content have significantly higher crude fat content; in the variety Ákos mentioned above the crude fat content is 16.7% while in the one designated with BS-56, it is 20.4%. The same was found for the foreign varieties; the variety Bora (Germany) contained 38.2% crude protein and 18.5% crude fat. The highest crude fat content (22.3%) was shown by Gadir (USA), combined with a crude protein content of 24.8%. Besides a 27.4% crude protein content the variety Ewans (USA) contained 21.3%

Table 2

Percentage chemical composition of extracted meals of various soybean varieties (in 86% dry matter content)

Designation of variety	crude protein	crude fat	ash
	content		
S 1346	39.6	0.2	5.5
Dawson	43.5	0.2	6.0
Cx-117	42.5	0.3	5.3
Cx-134	42.2	0.4	5.4
Ewans	34.5	0.5	7.0
Gadír	33.0	0.6	6.9
B-070	40.8	0.4	6.8
CM-048	36.0	0.4	7.7
S-1460	43.8	0.3	6.5
CM-137	34.7	0.3	7.4
10305-01	35.0	0.5	7.2
Bora	45.6	0.3	5.0
Bonanza	43.2	0.4	5.0
Chandor	37.9	0.4	5.6
Apache	36.3	0.6	7.0
KG-60	39.0	0.6	6.7
KG-30	43.6	0.4	6.5
NS-16	41.9	0.3	6.5
$\bar{x}$	39.6±3.9	0.4±0.1	6.3±0.8
ISz-15	35.6	0.4	6.2
ISz-16	37.3	0.4	6.9
Gate M-511	40.7	0.4	5.8
Éva	40.6	0.6	6.2
Ákos	46.3	0.3	5.4
Bolero	41.6	0.3	5.5
BS-44	35.7	0.7	7.3
BS-45	33.3	0.6	7.8
BS-31	35.5	0.5	7.7
BS-56	33.1	0.3	7.1
BS-38	37.5	0.5	8.1
BS-247	36.2	0.2	7.9
$\bar{x}$	37.7±3.8	0.4±0.1	6.8±0.9

crude fat. The crude fat content of the variety B-070 (USA) is 16.6%, the lowest among the foreign varieties. Both the Hungarian and the foreign varieties showed about 25% difference in crude fat, and some 35% difference in crude protein content.

The crude fibre content showed a 57% difference; the lowest amount of crude fibre (3.6%) was found in the variety Bonanza (Germany) and the highest (8.4%) in CM-048 (USA). The crude fibre content of the Hungarian varieties ranged between 4.3 and 8.0%. The crude fibre content was 5.6 ± 1.3 in the foreign varieties and 6.1 ± 1.2 in the Hungarian ones.

The lowest value of the ash content was 4.4%, and the highest one, 5.7%. In this case, the difference between the varieties reached 23%.

Table 3

Amino acid composition of various extracted soybean varieties as percentages of crude protein (g/16 g N)

Designation of variety	Threonine	Cystine	Valine	Methionine	Isoleucine	Leucine	Tyrosine	Phenyla-lanine	Lysine
S 1346	4.4	1.1	4.1	0.8	3.6	6.3	3.3	4.5	6.2
Dawson	3.9	1.0	4.7	0.8	5.2	8.3	2.9	6.6	6.1
Cx-117	4.3	1.3	4.7	0.9	4.1	7.9	3.7	5.2	6.2
Cx-134	4.2	1.1	4.6	1.2	4.0	8.2	3.3	5.7	6.5
Ewans	5.1	1.4	4.6	1.5	4.4	7.6	2.9	5.0	6.4
Gadír	5.4	1.9	4.9	1.3	4.5	7.8	4.1	5.5	6.8
B-070	4.5	1.4	4.8	1.1	4.5	6.8	3.5	4.9	5.8
CM-048	4.5	1.6	4.5	1.3	4.2	7.9	3.8	4.8	6.8
S-1460	4.0	1.4	4.5	1.0	4.1	7.1	3.3	5.3	5.1
CM-137	4.4	1.1	4.8	1.3	3.9	7.7	4.1	5.2	5.6
10305-01	4.4	1.3	4.6	1.1	4.0	7.7	3.7	5.1	5.2
Bora	4.5	1.4	5.2	1.0	5.5	8.0	4.1	5.1	7.5
Bonanza	4.1	1.3	4.8	1.1	4.1	7.9	3.7	5.6	7.2
Chandor	4.1	1.0	4.8	0.8	4.5	7.1	3.7	5.4	6.4
Apache	4.0	1.3	5.1	1.8	3.8	7.8	3.5	5.3	6.7
KG-60	4.1	1.3	4.3	1.0	3.8	7.1	4.3	4.8	4.9
KG-30	5.0	1.7	4.9	1.3	4.9	7.8	3.9	5.5	5.8
NS-16	3.9	1.2	4.6	1.2	4.4	6.5	3.6	4.8	5.3
$\bar{x}$	4.4 ± 0.4	1.3 ± 0.2	4.7 ± 0.2	1.1 ± 0.2	4.3 ± 0.4	7.5 ± 0.5	3.6 ± 0.3	5.2 ± 0.4	6.1 ± 0.7
ISz-15	4.9	1.3	5.6	1.4	4.5	5.9	3.9	5.5	7.6
ISz-16	4.3	1.5	5.0	1.4	3.9	7.5	3.6	4.8	5.5
Gate M-511	3.8	1.0	4.8	1.1	4.1	7.4	3.0	4.6	7.0
Éva	4.0	1.0	4.4	1.1	4.3	6.8	3.1	5.0	5.5
Ákos	4.5	1.1	4.5	1.2	4.4	7.3	3.6	4.9	5.7
Bolero	4.4	1.2	4.4	1.2	4.2	7.0	3.4	5.0	6.5
BS-44	4.7	1.1	5.0	1.0	4.1	7.3	3.8	4.8	5.9
BS-45	4.1	1.1	4.7	1.1	4.2	7.4	3.9	5.0	5.7
BS-31	4.4	1.5	4.7	1.2	3.8	7.5	4.2	5.0	5.4
BS-56	4.8	1.4	5.0	1.4	3.8	6.2	4.5	5.2	5.6
BS-38	4.5	1.2	4.6	1.2	4.1	7.1	4.4	4.8	4.7
BS-247	4.3	1.2	4.5	1.3	3.4	8.1	3.7	4.9	6.3
$\bar{x}$	4.3 ± 0.3	1.2 ± 0.1	4.7 ± 0.3	1.2 ± 0.1	4.0 ± 0.3	7.1 ± 0.5	3.7 ± 0.4	4.9 ± 0.2	5.9 ± 0.7

Table 4

Amino acid composition of various extracted soybean varieties, %
(in terms of 86% dry matter content)

Designation of variety	Threonine	Cystine	Valine	Methionine	Isoleucine	Leucine	Tyrosine	Phenyl-alanine	Lysine
S 1346	1.73	0.44	1.62	0.30	1.43	2.48	1.30	1.79	2.46
Dawson	1.71	0.44	2.05	0.34	2.25	3.59	1.28	2.85	2.66
Cx-117	1.81	0.57	2.01	0.38	1.76	3.38	1.59	2.21	2.63
Cx-134	1.77	0.45	1.92	0.53	1.69	3.47	1.41	2.41	2.75
Ewans	1.77	0.49	1.58	0.53	1.51	2.62	1.27	1.74	2.22
Gadír	1.79	0.61	1.62	0.42	1.50	2.57	1.34	1.80	2.25
B-070	1.85	0.58	1.95	0.45	1.82	2.76	1.44	1.99	2.38
CM-048	1.62	0.56	1.63	0.47	1.52	2.83	1.37	1.73	2.46
S-1460	1.77	0.61	1.98	0.42	1.79	3.11	1.46	2.30	2.23
CM-137	1.51	0.39	1.65	0.44	1.35	2.68	1.41	1.79	1.95
10305-01	1.54	0.45	1.62	0.37	1.41	2.71	1.29	1.78	1.82
Bora	2.06	0.63	2.39	0.45	2.49	3.65	1.85	2.32	3.42
Bonanza	1.76	0.58	2.06	0.47	1.78	3.41	1.59	2.40	3.11
Chandor	1.56	0.37	1.83	0.32	1.69	2.67	1.40	2.04	2.41
Apache	1.44	0.48	1.85	0.64	1.39	2.85	1.27	1.91	2.42
KG-60	1.59	0.51	1.66	0.40	1.50	2.77	1.69	1.86	1.92
KG-30	2.17	0.73	2.13	0.55	2.12	3.39	1.71	2.39	2.53
NS-16	1.63	0.52	1.93	0.49	1.85	2.71	1.51	2.00	2.21
$\bar{x}$	1,73 ± ±0.18	0.52 ± ±0.09	1.86 ± ±0.22	0.44 ± ±0.08	1.71 ± ±0.31	2.98 ± ±0.39	1.45 ± ±0.17	2.07 ± ±0.31	2.43 ± ±0.39
ISz-15	1.78	0.48	2.01	0.51	1.61	2.13	1.39	1.99	2.75
ISz-16	1.62	0.55	1.87	0.52	1.46	2.82	1.36	1.77	2.06
Gate M-511	1.54	0.41	1.95	0.46	1.68	3.01	1.23	1.89	2.86
Éva	1.64	0.42	1.77	0.46	1.75	2.75	1.28	2.02	2.24
Ákos	2.09	0.50	2.08	0.53	2.04	3.39	1.68	2.29	2.62
Bolero	1.82	0.51	1.82	0.51	1.73	2.93	1.42	2.08	2.71
BS-44	1.67	0.39	1.79	0.36	1.46	2.60	1.36	1.72	2.10
BS-45	1.40	0.37	1.57	0.37	1.40	2.45	1.30	1.65	1.91
BS-31	1.55	0.55	1.66	0.44	1.34	2.68	1.49	1.78	1.91
BS-56	1.60	0.47	1.64	0.46	1.27	2.05	1.48	1.73	1.84
BS-38	1.68	0.44	1.71	0.44	1.53	2.65	1.64	1.79	1.77
BS-247	1.56	0.43	1.61	0.44	1.24	2.95	1.34	1.79	2.28
$\bar{x}$	1.66 ± ±0.17	0.46 ± ±0.05	1.79 ± ±0.16	0.46 ± ±0.05	1.54 ± ±0.23	2.70 ± ±0.37	1.41 ± ±0.13	1.87 ± ±0.18	2.25 ± ±0.38

The N-free extracted matter was 23.9 ± 3.6% in the foreign soybeans and 23.8 ± 2.6% in the Hungarian varieties.

In Table 2 can be seen the chemical composition of the extracted and heat-treated meals. As shown by the data the extraction was successful, the crude fat content of the meals ranges from 0.2 to 0.7%. The dry matter content of the extracted soybean meals was found to be between 89.2 and 91.7%, which slightly exceeds the corresponding value of the commercial meals.

In regard to crude protein content, the best varieties proved to be Bora, Dawson, Bonanza, Ákos, S-1460 and KG-30 (43.2—46.3%), while ISz-15, Apache, Ewans, Gadir, BS-45, BS-31, 10305-01, BS-38, CM-048, BS-56 and CM-137 contained the least crude protein (33.0—36.3%). The crude protein content of the

foreign varieties was 39.6±3.9%, and that of the Hungarian soybean meals, 37.7±3.8%. The crude fat content of both the Hungarian and the foreign soybeans was 0.4±0.1%.

The quality of proteins is characterized by their amino acid composition. Tables 3 and 4 show the amino acid composition of the extracted soybean meals in terms of both protein and dry matter percentage.

Table 3 lists the 9 amino acids (g amino acid/16 g N) most important for the monogastric animals, as grouped by foreign and Hungarian soybean varieties. The lysine content is 6.1±0.7 in the foreign and 5.9±0.7 g/16 g N in the Hungarian varieties; the highest values were obtained in the German varieties Bora and Bonanza (7.2—7.5 g) and in the Hungarian variety ISZ-15 (7.6 g), while the lowest ones were measured in KG-60 (Canada) and BS-38 (Hungary) (4.9 g/16 g N and 4.7 g, respectively). Methionine and cystine together were 2.4±0.2 g/16 g N in the foreign and Hungarian varieties alike. The lowest methionine content (0.8 g/16 g N) was found in the varieties S-1346 and Dawson (USA) and in Chandor (France); among the Hungarian varieties BS-44 gave the lowest value (1.0 g/16 g N). The highest methionine content (1.5 g/16 g N) was obtained in Ewans (USA), and among the Hungarian varieties in ISZ-15, ISZ-16 and BS-56 (1.4 g/16 g N). The cystine content was lowest (1.0 g/16 g N) in Dawson (USA) and Chandor (France), and in the Hungarian varieties Gate-M-511 and Éva. The highest cystine content (1.7—1.9 g/16 g N) was measured in KG-30 (Canada) and Gadir (USA), respectively, and in the Hungarian variety BS-31 (1.5 g/16 g N).

The threonine content was practically the same in the foreign and Hungarian varieties (4.40±0.4 and 4.30±0.3 g/16 g N, respectively). The lowest value 3.9 g/16 g N) was found in Dawson (USA) and NS-16 (Yugoslav), and in the Hungarian variety Gate-M-511 (3.8 g/16 g N); the highest threonine content was determined in the varieties KG-30 (Canada) and Ewans and Gadir (USA) (5.0 and 5.4 g/16 g N, respectively), and in the Hungarian varieties BS-56 and ISZ-15 (4.8 and 4.9 g/16 g N, respectively).

Although the value of standard deviation of amino acid content is rather low in the foreign and Hungarian soybean varieties, the difference between the lowest and highest value of e.g. lysine content still exceeds 50%.

In Table 4, the 9 amino acids most important for feeding are given in terms of 86% dry matter content, grouped again by foreign- and Hungarian bred varieties. The lysine content of extracted meals is 2.43±0.3% for the foreign varieties, and 2.25±0.38% for the Hungarian ones; the difference in methionine content between the two groups is minimal (0.44±0.08 and 0.46±0.05%, respectively); the cystine content is 0.52±0.09% in the foreign and 0.46±0.05% in the Hungarian varieties; the threonine content is 1.73±0.18% in the foreign- and 1.66±0.17% in the Hungarian bred soybeans.

In the amino acid composition of proteins in the extracted soybeans, significant differences were found for both the Hungarian and the foreign varieties. With the Hungarian and foreign varieties jointly considered, the difference is 4% in lysine-, 8% in cystine- and 9% in methionine content on the average, while the average value of threonine content is the same for the foreign and Hungarian varieties.

Table 5

Major protein utilization parameters of extracted meals of various soybean varieties on the basis of N-metabolism examinations with rats

Designation of variety	Daily body mass gain, g	Daily N-balance, mg	Protein			
			biological value	true digestibility	net utilization	productive utilization
			%			
S 1346	2.6	70	79.9±1.7	81.6±5.1	65.2±5.4	43.5±5.4
Dawson	2.2	57	78.5±3.6	80.7±3.6	63.4±5.1	39.3±5.1
Cx-117	2.2	52	68.0±6.7	84.6±2.4	57.6±6.8	34.5±6.8
Cx-134	3.0	62	69.3±3.6	87.9±1.8	60.9±2.9	39.0±3.5
Ewans	3.0	66	81.1±4.7	81.3±2.8	65.9±3.5	43.2±3.2
Gadír	2.4	62	79.8±3.6	75.4±2.0	60.1±2.6	38.4±2.6
B-070	2.0	62	75.6±9.2	80.7±3.4	60.8±6.2	38.7±6.3
CM-048	2.6	71	82.0±1.9	80.9±2.1	66.4±2.8	44.5±2.8
S-1460	1.8	64	74.9±4.0	88.2±2.4	66.0±3.6	42.7±3.6
CM-137	2.2	66	83.7±3.6	79.7±3.5	66.8±5.3	43.4±5.3
10305-01	1.0	63	78.9±2.8	78.7±2.8	62.1±3.5	40.0±3.6
Bora	2.4	60	71.8±2.6	84.7±5.0	60.7±3.5	38.5±3.5
Bonanza	3.4	54	66.2±4.4	88.3±2.7	58.4±2.8	35.5±2.7
Chandor	2.6	56	74.2±5.9	78.5±5.6	58.0±4.4	35.6±4.4
Apache	2.6	61	81.4±5.3	73.7±8.3	60.0±7.7	38.1±7.7
KG-60	2.2	67	78.1±2.3	81.1±4.2	63.3±3.9	41.6±3.9
KG-30	2.4	69	78.4±2.2	83.0±3.5	65.0±2.2	43.1±2.2
NS-16	1.8	56	73.1±2.1	83.0±3.4	60.7±3.0	37.3±3.0
$\bar{x}$	2.3±0.5	62±5.5	76.4±5.0	81.8±4.0	62.2±3.0	39.8±3.0
ISz-15	2.4	62	76.4±3.7	78.8±1.8	60.1±2.5	38.4±2.4
ISz-16	2.6	68	79.6±3.4	82.7±4.3	65.8±3.3	43.3±3.2
Gate M-511	2.6	63	76.1±3.7	79.3±3.9	60.2±3.2	38.8±3.2
Éva	2.6	74	80.1±4.4	85.5±3.7	68.4±2.8	46.4±2.8
Ákos	2.2	61	70.2±5.3	84.4±3.3	59.1±2.4	37.7±2.4
Bolero	2.2	71	75.0±5.2	84.5±2.3	63.3±3.6	42.4±3.6
BS-44	1.6	62	83.7±2.8	76.9±7.8	64.4±6.4	51.2±6.5
BS-45	2.6	74	88.4±3.7	78.8±1.7	69.7±4.1	47.3±4.1
BS-31	2.4	70	84.5±7.5	82.2±3.8	69.7±8.4	46.5±8.3
BS-56	2.2	60	81.9±2.5	77.2±4.7	63.2±3.1	39.9±3.1
BS-38	2.2	65	86.3±2.5	75.3±4.9	64.9±2.9	42.2±2.9
BS-247	2.0	56	81.2±5.3	74.9±3.1	61.0±6.2	37.6±6.1
$\bar{x}$	2.3±0.3	65±5.8	80.2±5.2	80.0±3.7	64.1±3.7	42.6±4.4

Experiments with rats

The extracted soybeans used in the rat experiments were checked by the above described "TI"-test, and their heat treatment was found to be satisfactory (TIU mg < 10).

The protein utilization parameters obtained, on the basis of the data of N-metabolism tests performed with young rats, are given in Table 5. In addition, the daily weight gain and N retention of the animals are also contained in the table.

With the Hungarian soybeans 2.0—2.6 g, with the foreign ones 1.6—3.4 g daily weight gain was attained. Thus, the influence of the foreign varieties on the body mass gain showed a much wider fluctuation than that caused by the Hungarian varieties. It is to be noted, however, that even the 2.4 g daily body mass increase exceeds the usual values of extracted soybean samples of average quality. This suggests that the heat treatment was satisfactory and the effect of the trypsin inhibitor was reducer to a minimum.

The daily N-balance was 56—74 mg with the Hungarian soybean varieties and 52—71 mg with the foreign ones. The difference between the extreme values is in both cases about 34%.

The biological value of proteins in the extracted soybean meal is 70.2—88.4% with the Hungarian varieties and 66.2—83.7% with the foreign soybeans; the difference between the extreme values is about 25%. The average biological value is 80.2 (sd: 5.2) for the Hungarian varieties and 76.4 (sd: 5.0) for the foreign ones; the difference is not significant.

The true digestibility of the extracted soybean proteins can be considered the same ($80.0 \pm 3.7\%$ and $81.8 \pm 4.0\%$, respectively).

The lowest net protein utilization (57.6%) was obtained with the variety Cx-117 (USA) while the highest one (69.7%) with the Hungarian varieties BS-31 and BS-45; its average value is 64.1 (sd: 3.7) for the Hungarian varieties and 62.2 (sd: 3.0) for the foreign ones.

The productive utilization of proteins is 42.6 ± 4.4 with the Hungarian varieties and 39.8 ± 3.0 with the foreign ones; the difference is not significant. The lowest value (34.5%) was obatined with Cx-117 (USA), the highest one (47.3%) with the Hungarian variety BS-45.

On the basis of the N-metabolism tests performed with rats it can be established that the difference in protein utilization parameters between the Hungarian and foreign varieties is minimal.

Conclusions

The comparative examination of foreign and Hungarian soybean varieties grown in the same year at the same site was carried out with chemical and biological methods. The inactivation of the trypsin inhibitor was proved in our experiment not only by the TI-test, but also by the good appetite and body mass gain shown by the rats during the metabolism examination. Zelter (1971) e.g. did not always find the urease test reliable and suggested taking the biological effect on the animals for a basis.

According to the protein utilization parameters established by chemical analyses and animal experiments, significant differences in seed production between the foreign and the Hungarian soybean varieties could not be found (at $P = 0.01$). The values obtained in our chemical analyses for the nutrient content, and for the amino acid composition in particular, are within the results obtained abroad and in Hungary in similar examinations. The results of metabolism experiments carried out with rats do not exceed the limits of value of similar tests performed abroad

(investigations of this nature are not continued in Hungary). We also examined the possible correlations between the various nutritive elements, and found a negative correlation between the crude protein- and the crude fat content of soybeans ($r = -0.764$ for the Hungarian, and $r = -0.512$ for the foreign varieties). This correlation is not as close as the one determined by Mieth et al. (1988).

Between the crude protein- and the lysine content of the extracted soybean meals a correlation of $r = 0.700$ for the foreign and of $r = 0.646$ for the Hungarian varieties was found, while the correlation between crude protein- and threonine content was $r = 0.588$ for the foreign varieties and $r = 0.740$ for the Hungarian ones.

Negative correlation was found again between the crude protein content of the extracted soybean meals and the biological value determined by experiments with rats ($r = -0.726$ for the foreign and $r = -0.793$ for the Hungarian varieties). This seems to confirm the earlier statement that increases in the protein content do not generally coincide with improvement in the protein quality. Between the crude protein content and the true digestibility of protein, on the other hand, the correlation is positive (foreign varieties: $r = 0.735$, Hungarian varieties: $r = 0.642$).

The importance of the above correlations is emphasized by the fact that the 30 soybean samples originated from the same year and growing site. Thus, we had no need to take the climatic- and soil conditions into consideration when comparing the varieties.

These results call attention to the fact that the protein content of the soybean and the amino acid composition within, as well as the utilization of protein in the animal organism, range between wide limits, to which attention should by all means be paid when the variety is chosen.

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CORRELATION BETWEEN IODINE SUPPLY AND PROTEIN UTILIZATION

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To study the correlation between iodine supply and protein utilization, the authors carried out metabolism experiments with rats and pigs. The rat experiments were conducted with 5 groups—4 experimental and one control group—with 10 animals per group. The iodine supplements were 0.3, 0.6, 0.9 and 1.2 mg/kg dry matter, respectively.

The pig experiments were carried out with 4 animals in 7 periods, with one control period both at the beginning and in the end of the experiments; the rate of iodine completion in terms of dry matter was: 0.3, 0.6, 0.9, 0.6 and 0.3 mg/kg, respectively.

According to the results of the experiments with pigs, the digestibility of protein and lysine significantly increased in response to iodine supplement, and the rat experiments showed a similar though lower degree tendency. The concentration of thyroxines (T_3 and T_4) did not practically change under the influence of iodine supply.

Keywords: iodine supply, protein, utilization, digestibility

Introduction

According to literary data the iodine requirement for livestock ranges from 0.1 to 0.8 mg/kg feed dry matter (Groppel, 1983) depending on the temperature of the environment, the development stage of the animals, and above all on the thyreostatic substances present in the feedstuffs.

In the absence of substances inhibiting the iodine metabolism, the iodine demand of growing cattle and swine can be satisfied with 0.15—0.20 mg/kg iodine in the dry matter of feed, that of pregnant and suckling animals with 0.20—0.30 mg/kg, while in the presence of thyreostatic substances the requirements may increase two- or threefold.

Iodine is concentrated in the organism in three forms: as inorganic iodine in the blood plasma, in the thyroid gland, and hormonally or protein-bound in the plasma and tissues (Underwood, 1977; Prasad, 1978; Groppel and Körber, 1985). There is a continuous exchange between the thyroid gland and the extrathyroidal iodine poles. The thyroxines play a manifold role in metabolism. They take part in energy-, lipid-, carbonhydrate-, mineral and—last but not least—protein metabolism. We carried out experiments with rats and pigs to study the correlation between iodine supply and protein utilization.

Materials and methods

The N-metabolism experiments with rats were carried out in 5 groups, with 10 animals per group, in a control and 4 experimental groups, according to the method in the ÁTK (Szelényi, 1969). The iodine

Table 1

Treatment

	Metabolism experiments	
	Rat n=10	Pig n=4
I.		Control
II.	Control	0.3 mg/kg J
III.	0.3 mg/kg J	0.6 mg/kg J
IV.	0.6 mg/kg J	0.9 mg/kg J
V.	0.9 mg/kg J	0.6 mg/kg J
VI.	1.2 mg/kg J	0.3 mg/kg J
VII.		Control

supplement was 0.3, 0.6, 0.9 and 1.2 mg/kg dry matter of 0.3, 0.6 feed in group II, III, IV and V, respectively. The N-metabolism experiments with pigs were conducted in 7 periods, with 4 animals per period (Gundel and Babinszky, 1988) and a control period both at the beginning and at the end of the experiment (Table 1). The iodine supplement was 0.3 mg/kg dry matter of feed in group II and 0.6 mg/kg in group III and V, and 0.9 mg/kg in group IV; in periods I and VII the pigs received iodine-free feed. The iodine was supplied in the form of potassium iodate (KIO_3).

Results

According to the results of metabolism experiments with pigs (Table 2), under the influence of different (increasing then decreasing) extents of iodine supply, and the apparent digestibility of crude protein significantly—8.2—11.5%—increased compared to control I.

According to the technology of metabolism experiments employed in our institute, through 7 periods of the experiment involved the same young pigs. The difference (8.1%) between the coefficients of protein digestion in the control phases I and VII is the consequence. Therefore, since the organism of the animals sup-

Table 2

Effects of different iodine supplements on the apparent digestibility of crude protein and lysine in pigs

	Digestion coefficients %	
	Crude protein	Lysine
Control	80.3	84.6
Iodine supplements		
0.3 mg/kg	91.8***	90.8***
0.6 mg/kg	89.7**	92.4***
0.9 mg/kg	88.5**	93.3****
0.6 mg/kg	90.3***	92.0****
0.3 mg/kg	90.9***	87.7***
Control	88.4**	85.8

**=P<5% ***=P<1% ****=P<0.1%

posedly stored some of the iodine supplied in the previous phases, their iodine status compared to the control phase one, they were the better.

The digestibility of lysine, like that of protein, significantly increased in response to iodine supply. The two curves in Fig. 1 show the trend of digestibility of protein and lysine.

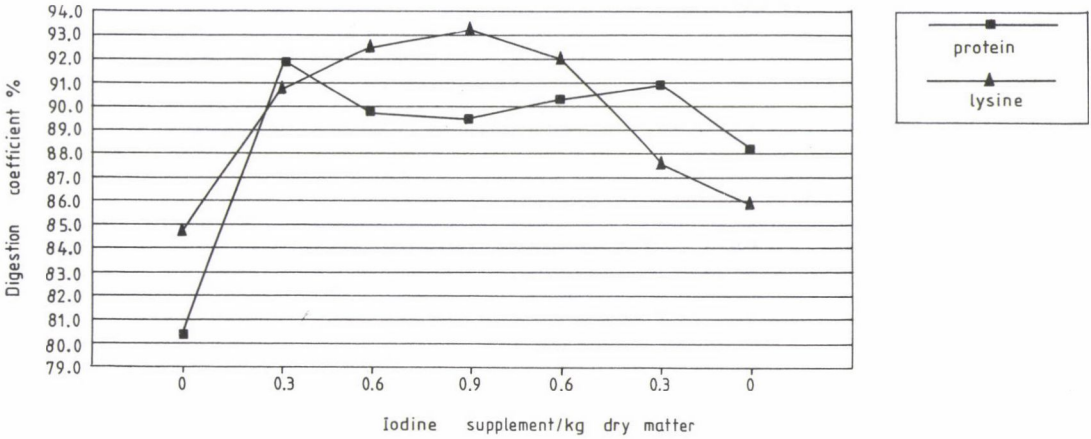

Fig. 1. Effect of iodine supply on the digestibility of protein and lysine

The results of the experiments with rats confirm the metabolism tests on pigs. In response to iodine supplements, the N-balance rose to 64 mg compared to 57 mg in the control, the biological value of the feed consumed increased slightly, the actual digestibility (Table 3) of protein from 71.2% to 75.7%, and the net and productive protein utilization also improved. In the end of the rat experiments, the T_3 and T_4 hormone concentration of the blood was also determined (Table 4). The concentration of the T_4 hormone was between 41.8 ± 9.7 μmol/l and 31.5 nmol/l, while that of the T_3 hormone ranged from 4.4 nmol/l to 3.1 nmol/l. The

Table 3

Effects of iodine supplements on some protein utilization parameters in rat experiments, %

Iodine supplements, mg/kg		Protein			
		biological value	actual digestibility	net utilization	productive utilization
I.	—	85.3	71.2	60.7	37.8
II.	0.3	81.3	73.1	59.3	36.0
III.	0.6	86.0	73.9	63.6	40.3
IV.	0.9	87.0	73.7	64.1	40.8
V.	1.2	86.8	75.7	65.7	42.3

Table 4

T_3 and T_4 hormone concentrations in the blood of rats in response to different rates of iodine supply

Group, mg/kg J	T_4	T_3
	hormone concentration, μmol/l	
1. Control	41.8± 9.7	4.4±0.6
2. 0.3	42.6±17.5	4.2±0.7
3. 0.6	35.3± 9.9	3.2±0.6
4. 0.9	36.4±10.9	3.3±1.0
5. 1.2	31.5± 5.5	3.1±0.1

normal T_4 hormone concentration in the blood of rats is between 32.2 and 51.5 nmol/l, while that of the T_3 hormone is 2.3—5.4 nmol/l. Thus, the data obtained in our experiments are within the normal limits of value, and the iodine supply did not cause essential changes.

Discussion

The iodine is an essential component of the thyroxines (T_3—T_4) so its physiological effect cannot be separated from the role of the thyroid gland and its hormones. Soon after its discovery by Courtois (1813) iodine was used (Coindet, 1820) in the treatment of goitre, and then the iodine deficiency of drinking water was pointed out (Chatin, 1851) to be the cause of goitre, an endemic disease in iodine-deficient regions.

The development of goitre is most often caused by the primary of secondary iodine deficiency, which may occur among livestock and even in humans. As a consequence of insufficient iodine supply, lesions of hair and skin, reproduction disorders, reduced performance, etc., and in man intellectual retardation, may appear beside goitre formation (Groppel and Körber, 1985).

Through the thyroxines the iodine plays an important role in the protein metabolism (Müller, 1982; Stebodzinski, 1981), as proved by the results of metabolism tests performed on pigs, namely, in response to iodine supply the apparent digestibility of crude protein significantly increased.

The best results were obtained with the 0.3 mg/kg iodine supplement, which confirms the data of Anke (1982) and Groppel and Körber (1985). Accordingly, without thyreostatic substances the iodine demand of pigs is 0.2—0.3 mg/kg dry matter of feed.

The results of experiments with rats suggest that the effect of iodine supply on protein utilization in rats is lower than in the case of pigs. Bergner (1986) arrived at similar conclusions when giving thyroxines (T_3 and T_4) instead of iodine in experiments with rats and pigs; he found that the protein, lysine and leucine synthesis greatly increased, but the extent of the increase was much higher in pigs than in rats.

As a result of iodine supply, the T_3—T_4 values obtained in the serum of rats were within the normal level. It is known that 90% of the iodine present in the organism is bound to protein (Asplund et al., 1959; Blödow, 1969). In the serum the inactive protein-bound and the active free thyroxines are equally present. Groppel (1986) found in experiments that the iodine supply influenced the values of T_3 and T_4 in the blood serum only after a long time. In our rat experiments we followed the method of metabolism experiments; their period is not long enough for the iodine supply to cause any reliable change in the concentration of the thyroxines. In the case of iodine deficient feeding too, the T_4 content, then the T_3 concentration only decreased in the serum after the depletion of the iodine reserves of the thyroid gland (Groppel, 1986). Further literary data (Körber, 1983; Gürtler et al., 1983; Pethes et al., 1983) prove the correlation between T_4 and protein-bound iodine, and point out that the iodine supply increases the level of thyroxines only in animals permanently kept on iodine-deficient diet and having exhausted the iodine reserves. In other cases the differences obtained are accidental, which supposedly is the case in our present experiments too.

As a consequence of iodine-deficient feeding, the uptake of feed may decrease, which naturally has a negative influence on all phases of animal production.

Table 5

Extreme values of iodine contents in various feedstuffs (μg/kg)

Maize grain	29— 60
Wheat grain	40— 110
Barley grain	35— 95
Bran	70— 250
Extracted rape meal	100— 120
Extracted soymeal	70— 100
Fishmeal	3450—8250
Rape (whole plant)	185— 270
Grass	80— 150
Silage maize	190— 430
Red clover	180— 270
Alfalfa	190— 350
Beet greens	250— 590
Wheat straw	140— 310

The iodine content of feedstuffs used in the practice of pig feeding (Table 5) is so low—except in fishmeal—that iodine supplements are in every case necessary for an optium production.

The potassium iodide which is absorbed quickly, and in high proportion, is an excellent supplement, but has the disadvantage that it is less stable than the potassium iodate.

Under unfavourable conditions or in the case of compound feed stored for a long time, a part of the iodine may escape (Groppel, 1986), which may result not

only in reduced production, due partly to the lower protein conversion; but in the case of intensive pig production, the increased N discharge may even be a stress factor for the environment.

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Reviews

BIOTECHNOLOGY OF RAPESEED (*BRASSICA NAPUS* L.)

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The agronomic advantages of rapeseed biotechnology such as intergeneric hybridization through embryo rescue, protoplast fusion, haploid induction, somaclonal variation, mutant selection for herbicide resistance and transformation are summarized in this review. *Brassica napus* is the most important oil crop of the northern hemisphere, requiring, in its breeding programmes, all the biotechnology reported in this paper.

Keywords: *Brassica*, plant regeneration, mutant selection, transformation, embryo culture, haploid induction

Introduction

Rapeseed is one of the most successfully manipulated plant species in biotechnological research with, in addition, numerous results applied in practice (Glimelius et al., 1990; Trail et al., 1989).

As an oil crop, rapeseed has great importance in countries with an oceanic and subarctic climate of the northern temperate zone, where sunflower and soybean cannot be grown. In these areas rapeseed is the only oil crop besides flax. In Hungary, rapeseed plays an important role as an oil-producing plant for industrial and food purposes.

The most important tasks of biotechnology in rapeseed breeding are the following:

— *in vitro* selection of lines resistant pathogenes, pests and stress (Agnihotri et al., 1990)
— increase of genetic resources in *B. napus* by interspecific crosses, or by hybridization for combining advantageous characteristics of spring and winter rapeseed (Glimelius et al., 1990)
— production of cultivars, for vegetable oil purposes, containing less than 2% erucic acid, less than 30 μmole glucosinolate (00-type), less than 3% linolenic acid, and more than 30% linoleic acid (Trail et al., 1989; Chen et al., 1988; Pleines and Friedt, 1989; Eöri, 1986)
— production of cultivars with high linolenic and erucic acid content for industrial use (Fig. 1).

It has been found that fatty acid mutant selection is possible as a result of its linear metabolic pathway. Within the last decades zero-erucic acid ($C_{22:1}$) vari-

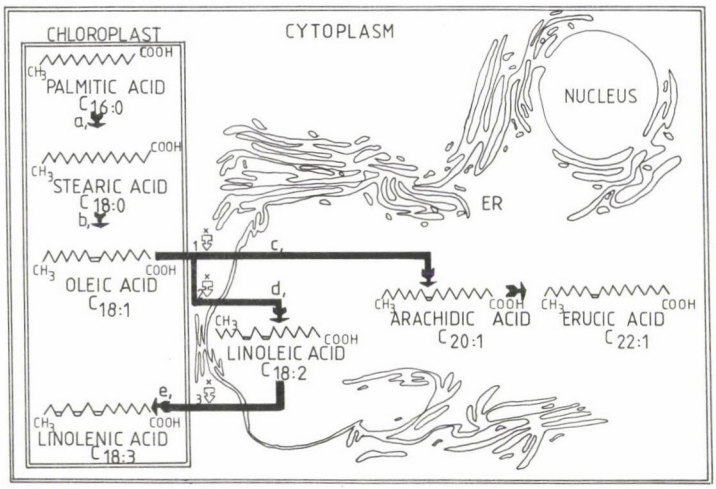

Fig. 1. Compartmentalization and biosynthetic pathway of fatty acids (Röbbelen 1971, Gyulai and Lehoczky 1986, Pleines and Friedt 1988). Arrows with star sign the possible points to block the elongation (**1x**) and desaturation (**2x, 3x**) of fatty acids, resulting in a rapeseed mutant with desired fatty acid content. Abbreviations: **ER** = endoplasmic reticulum, **a** = palmitoyl-ACP elongase, **b** = stearyl-PC desaturase, **c** = arachidoyl-ACP elongase, **d** = oleoyl-PC desaturase, **e** = linoleoyl-PC desaturase (designed by K. Ungár and G. Gyulai)

aties have been achieved. Siebel and Paul (1989) confirmed that the inheritance of erucic acid is determined by two genes which show no dominance and act in an additive fashion. It was also determined that multiple alleles (at least five) occur at each locus in *Brassica napus*. Further progress has been made to achieve high linoleic/low linolenic acid cultivars. New genotypes with these characters were induced by chemical mutagenesis (Röbbelen and Nitsch, 1975) and by interspecific hybridization (Fig. 2) with *B. juncea* (Roy and Tarr, 1987) and with low linolenic acid *B. napus* (Pleines and Friedt, 1989).

The values of fatty acid characters, such as ODR (Oleic Desaturation Ratio) and LDR (Linoleic Desaturation Ratio) in the new high linoleic/low linolenic acid varieties were between 17.3—43.8 and 8.2—20.9, respectively (Pleines and Friedt, 1988, 1989). The values of ODR and LDR were calculated as follows:

$$ODR = (\%C_{18:2} + \%C_{18:3})/(\%C_{18:1} + \%C_{18:2} + \%C_{18:3}) \times 100$$
$$LDR = \%C_{18:3}/(\%C_{18:2} + \%C_{18:3}) \times 100$$

The difficulties in establishing stable mutants or hybrids is that the expression of C_{18} fatty acids of both the dietary essential linoleic acid ($C_{18:2}$) and the oil self-oxidative factor of α-linolenic acid ($C_{18:3}$) is influenced not only by genotype but by the environment, particularly temperature during seed development (Pleines and Friedt, 1989).

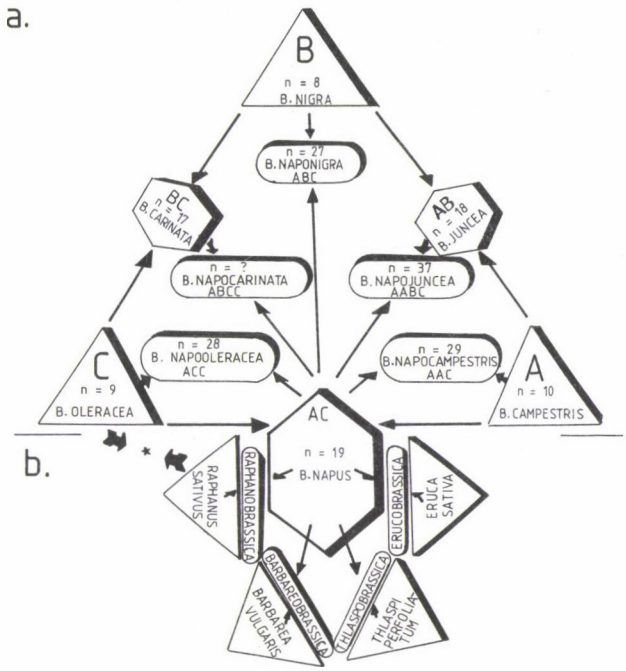

Fig. 2. Genomic relationship of *B. napus* (Morinaga 1934, U 1935), (**A**) and its interspecific hybrids (**B**), produced through protoplast fusion (Glimelius et al. 1990), and (*) by crossing (Karpecsenko 1927)

The effect of temperature on the activity dependence of the elongation and desaturation enzymes were as follows (Pleines and Friedt, 1988):

	22 °C : 96.1%	22 °C : 99.6%	22 °C : 26.0%	22 °C : 23.4%
	14 °C : 96.3%	14 °C : 99.8%	14 °C : 30.1%	14 °C : 31.2%
C16 : 0	→	C18 : 0 →	C18 : 1 →	C18 : 2 →	C18 : 3
	palmitoyl-ACP elongase	stearyl-PC desaturase	oleoyl-PC-desaturase	linoleoyl-PC desaturase	

I. Cell, tissue and organ culture

The establishment of plant regeneration systems is the prerequisite of the application of biotechnological methods. Similar to other *Brassicaceae* species (Table 1), *Brassica napus* belongs to the efficiently regenerating species (Glimelius et al., 1990). Plant regeneration has already been successful from all kinds of explants. Identical plants have been regenerated from calli induced from root, stem, leaf, cotyledon segments, anther and microspore cultures (in Sharma and Thorpe, 1989; Jain et al., 1988).

Table 1

The most important species of Brassica genus, chromosome numbers and genome constitutions (Morinaga 1934, U 1935, Murata and Orton 1987).

Latin name	Chromosome number	Genome constitution	cv	Common name	Hungarian name
B. campestris	2n = 2x = 20	(AA)	oleifera	turnip rape	réparepce
			narinosa	chinese savoy	—
			chinensis	chinese cabbage	kínai kel
			pekinensis	pe-tsai	—
B. nigra	2n = 2x = 16	(BB)	—	black mustard	fekete mustár
B. oleracea	2n = 2x = 18	(CC)	capitata	cabbage	fejes káposzta
			sabauda	savoy	kelkáposzta
			italica	broccoli	brokkoli
			botrytis	cauliflower	karfiol
			gemmifera	brussels sprouts	bimbós kel
			gongyloides	kohlrabi	karalábé
			oleifera	oil cabbage	káposztarepce
	2n = 4x = 36	(CCCC)	acephala	curly kale	takarmánykáposzta
B. juncea	2n = 4x = 36	(AABB)	—	brown mustard	szareptai mustár
B. napus	2n = 4x = 38	(AACC)	biennis	winter rape	őszi repce
			annua	spring rape	tavaszi repce
B. carinata	2n = 4x = 34	(BBCC)	—	abyssinian mustard	abesszin mustár
B. hirta	2n = 2x = 24	(SS)	—	white mustard	fehér mustár

An improved plant regeneration technique with the application of deplasmolised hypocotyl orginated protoplasts was reported by Dudits and Heszky (1990). In all these systems plant regeneration took place, after a dedifferentiated callus phase, through organogenesis or somatic embryogenesis (Fig. 3).

After comparison of the plant regeneration potential of the *Brassica* species, it was found that the highest rate of shoot regeneration capacity (20%) could be achieved with *B. napus* (AACC), while other amphidiploid species such as *B. carinata* (BBCC) and *B. juncea* (AABB) were regenerated with less efficiency (2.8%, and 1.2%, respectively).

From the plant regeneration of *B. oleracea* cv acephala (CCCC), it was assumed that the genome "C" carries the whole set of genes responsible for morphogenesis (Murata and Orton, 1987), while the genome "A" has a significant inhibitory effect on regeneration potential (Narasimhulu et al., 1988).

II. Resistant selection

Isolation of herbicide resistant plants was the first great result of biotechnology (Maliga et al., 1975). However, it was a general problem that the yield of herbicide resistant mutants failed to match the yield of non-resistant plants, even

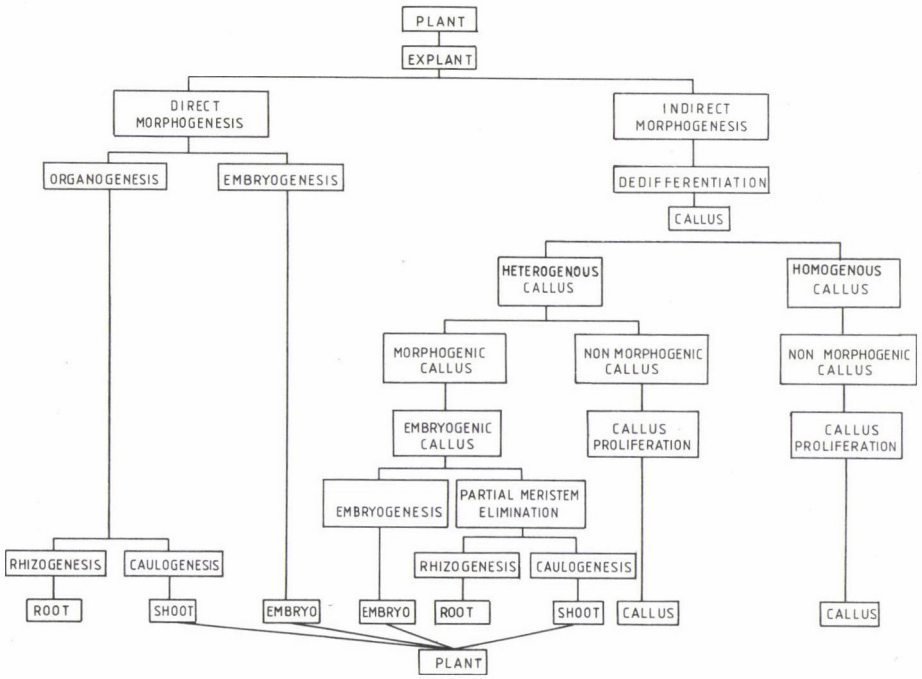

Fig. 3. Scheme of the alternative ways of *in vitro* morphogenesis (designed by L. E. Heszky, O. Toldi and G. Gyulai)

if these were strongly weed-infected. The average yield of *B. napus* can decrease by 10—15% as a consequence of weed infection.

The first atrazin resistant *B. napus* cv canola (Canola Council of Canada) varieties could not spread because of their reduced seed production (Swanson et al., 1988). A solution was found by classical backcross experiments, when triazin resistance from *B. campestris* L. could be transferred into the spring *B. napus* line (*B. napus* OAC Triton). This line is grown in several thousands of acres in Canada (Hatzios, 1987).

Isolation of chlorsulfuron resistant rapeseed lines is successfully progressing, based upon the reduction of sensibility of acetolactate synthetase (ALS) enzyme (Swanson et al., 1988). Selection for salt tolerance (see the general aims in Binh et al., 1992) by detection of proline accumulation has proved successful at callus level in rapeseed (Chandler and Thorpe, 1987).

A stable *Alternaria brassicola* resistant winter rapeseed line was selected from haploid callus culture (MacDonald et al., 1989) as a result of irradiation and ethyl-methane-sulphonate treatment.

III. Gene transfer

III. 1. Interspecific and intergeneric somatic cell hybrids

Species of *Brassica* genus are very mobile genetically. Despite belonging to the very early domesticated species, rapeseed possesses a relatively narrow genetic capacity (Glimelius et al., 1990). Clearly defined genomic relations allow for directed gene transfer among *Brassica* species (Table 1). Therefore, numerous approaches have been attempted to widen the genetic variation in *Brassica napus* (Karpecsenko, 1927). Besides other genetic manipulations, somatic cell hybridizations experiments via protoplast fusion seem to be the most promising. The most important evolutionary connections and the interspecific and intergeneric hybrids of *Brassica napus* are summarized in Fig. 2.

Through via protoplast fusion, all three genomes of *Brassica* genus (A, B, C) were successfully combined (Fig. 2) in trigenomic *B. naponigra* (AABBCC), in amphitriploid *B. napooleracea* (AACCCC), in amphitetraploid *B. napojuncea* (AAAABBCC) and *B. napocarinata* (AABBCCCC) (Glimelius et al., 1990). The special significance of cell fusion hybridization in these cases that the *B. naponigra* could not have been produced by sexual crossing (Glimelius et al., 1990).

The aim of the production of intergeneric, parasexual hybrids (Fig. 2/B) is the transfer of drought and insect resistance from the *Eruca* species (*Erucobrassica*) on one hand, and CMS and Ogura type male sterility from *Raphanus* on the other, into the *Brassica napus* (Glimelius et al., 1990; Menczel et al., 1987).

For gene exchange and combination purposes among the members of the *Brassicaceae* family, the cold tolerant *Barbarea vulgaris* and the *Thlaspi perfoliatum* with 16% nervonic fatty acid ($C_{24:1}$) content seemed to be useful (Fig. 2). The testing of partial fusion hybrids with *B. napus* is now taking place (Glimelius et al., 1990).

From the standpoint of evolutionary genetics, the protoplast fusions between the *Brassica* species, or the resynthesis of *B. napus* via sexual crosses (see in Dudits and Heszky, 1990), may clarify the nuclear genome constitution of *B. napus* and the structure and relationship between chloroplast and mitochondrial plasmon in *B. napus* (Rosén et al., 1988).

The resynthesis of *Brassica napus* via interspecific hybridization of *B. campestris* and *B. oleracea* was successful (Sundberg et al., 1987).

III. 2. Direct gene transfer and Agrobacterium transformation

Agrobacterium transformation allows not only interspecific gene exchange, but also the transfer of the filogenetically far bacterial and animal genes into plants (Chilton et al., 1977).

Agrobacterium tumefaciens and *A. rhizogenes* carry special Ti and Ri plasmids, up to about 200 Kb in size. A segment of these plasmids of approximately 50 Kb (T-DNS) can be incorporated into the plant cell genome from the bacteria carrying the useful genes transformed previously into the plasmid (Chilton et al., 1977).

The transformation of stem segments (Fry et al., 1987), of hypocotyl segments of seedlings (Radke et al., 1988), of cotyledon pieces (Moloney et al., 1989) even of microspore cultures (Pechan, 1989) has already been successful, with the application of nopalin synthetase (Nop) as marker and β-glucuronidase (GUS) as reporter genes. All these results have created the technical possibility for producing transformant rapeseed lines with *Agrobacterium* plasmid carrying agronomically important genes. The cotyledon transformation system (Moloney et al., 1989) seems to be useful for the investigation of embryo specific gene expression, which may be connected with the oil storage and fatty acid metabolism in the seed.

The efficiency of first transformation experiments with *Agrobacterium rhizogenes* was low because of the great number of abnormal regenerants. As for the direct gene transfer, successful experiments in electroporation (Guerche et al., 1987) and microinjection (Spangenberg et al., 1986) were carried out in *B. napus* protoplast cultures.

IV. Embryo culture

Although far crosses among *Brassicaceae* species make it possible to transfer disease resistances and ecological tolerances, evolutionary hindrances limit the production of fertile hybrids. Early abortion of embryos of far hybrids can be avoided by embryo culture (Maróti, 1976; Dudits and Heszky, 1990).

The possibility of resynthesis of *B. napus* from the *B. campestris* × *B. oleracea* cross has been known since the thirties (Morinaga, 1934; U 1935) (Table 1).

With the help of embryo culture, the resynthesis of *B. napus* was successful by the crossing *B. campestris* with a special form of *B. oleracea*, namely *B. oleracea f. alboglabra* (Chen et al., 1988). This hybridization seemed to be the most useful for production of a special *B. napus* type (00-type) with a lower than 2% erucic acid and 30 µmole glucosinolate content, because a close correlation was found between toxic glucosinolate content on one hand, and fiber content and seed colour on the other. Fiber content is the lowest in the yellow seeded species and it increases in the brown and black seeded types. Crossing the yellow seeded *B. campestris* with the brown seeded *B. oleracea f. alboglabra* produced a yellow seeded *B. napus* (Chen et al., 1988).

B. campestris lines regenerated from the hybrid of *B. campestris* × *Eruca sativa* inherited the drought and *Albugo candida* tolerance of *Eruca sativa* (in Agnihotri et al., 1990). *B. campestris* derived from the hybrid embryo culture of *B. spinescens* × *B. campestris* showed the alternaria resistance of *B. spinescens* (Agnihotri et al., 1990).

An unsuccessful far (interspecific) hybridization was attempted for the transfer of vernalization genes from *B. napus* L. cv. Syoren Hakuran, an edible vegetable in Asia, into cabbage *B. oleracea* var. *capitata* L. cv. Joshin (Hossain et al., 1988). For synthetic seed production in rapeseed, cell suspensions of embryo cultures were synchronized by chemical agents at certain sites of the cell cycle (Fig. 4).

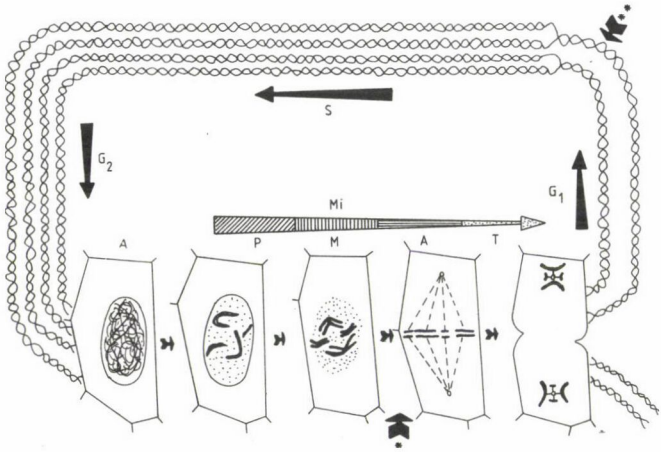

Fig. 4. Cell cycle with certain action sites for cell synchronization: at the metaphase (**x**) (e.g. colchicine, camphor) and in the S phase of DNA replication (**xx**) (e.g. hydroxyurea). **A** = anaphase, **G1** and **G2**—gap of 1 and 2 subphases of interphase, **M** = metaphase of mitosis, **Mi** = mitosis, **P** = prophase of mitosis, **S** = DNS synthesis subphase of interphase, **T** = telophase of mitosis

V. Haploid induction

Production of haploid plants is possible by means of the induction of embryogenesis from the microspore (anther and pollen culture) or from the macrospore (Dudits and Heszky, 1990).

The main purpose of these methods is the production of homozygous dihaploid lines necessary for mutant isolation and breeding programmes (Heszky and Mesch, 1976; Wenzel et al., 1977; Keller et al., 1987).

These homozygous lines are especially important in the case of vegetable species *B. campestris*, since the production of inbred lines meets difficulties because of self-incompatibility. Haploid induction plays a great role in erucic acid elimination of *B. napus*, for it has been proved by the crossings of dihaploid lines that erucic acid metabolism is controlled by two additive genes (Downey and Harvey, 1963; Pleines and Friedt, 1989).

The first haploid *B. napus* regeneration was achieved by Thomas and Wenzel (1975), but with very low efficiency. Optimizing the method (Fig. 5.) by:

1. conditioning the anther donor plants (weekly NPK fertilization; 10/15 °C night/day temperature, 16/8 hours photoperiod)
2. macroscopic recognition of the uninuclear stage of pollen (ratio of filament: anther = 1 : 3)
3. cold pretreatment of the buds (4 °C, 1 week in dark)
4. determination of the correct ratio of inoculated anthers and medium (6 anthers/6 ml solid or 3.5 ml liquid medium; in a microspore culture of pollen grains of 8 anthers/3 ml medium)

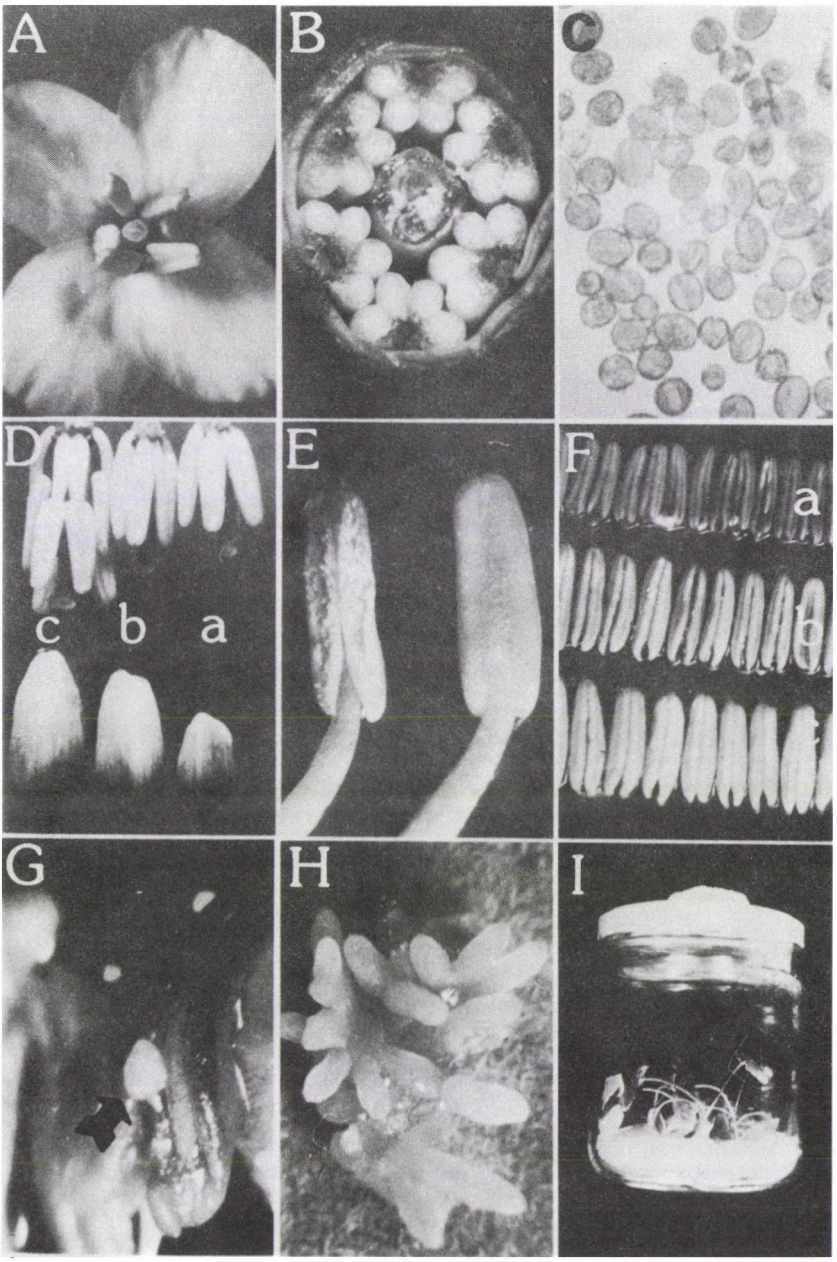

Fig. 5. Haploid induction in rapeseed (*Brassica napus* L.): (**A**) flower, (**B**) cross section of flower bud, (**C**) pollen culture, (**D**) size (a=3-, b=4-, c=5 mm) and the ratio between anthers and filaments, (**E**) adaxial and abaxial surfaces of the anther, (**F**) anther culture, isolated from the buds on Fig. **D,** (**G**) haploid embryogenesis at the globular stage, (**H**) adventive embryogenesis on the surface of the primary embryo, (**I**) and the regenerated haploid plants in shoot culture (experiments of Baricz A. and Gyulai G.)

5. using amino acids (glutamine, serine) as N-source and increased (12%) sucrose content, facilitates the haploid induction of *B. napus* which has been solved (Keller et al., 1987; Kameya and Hinata, 1970).

Haploids, regenerated from pollen and anther culture or rediploidized dihaploid lines, have already been successfully built into the breeding programme of *B. napus* (Siebel and Pauls, 1989).

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Book reviews

Strahlenschäden an Pflanzen. István Fendrik and Jenő Bors. Handbuch der Pflanzenkrankheiten, Band I. Die nichtparasitären Krankheiten (Handbook of plant diseases, Vol. I. Non-parasitic diseases) Part 6, 1, 1991, Paul Parey, Berlin—Hamburg, ISBN 3-489-76926-0, p. 208.

With this book another valuable section has been added to the multipartite series of "SORAUER", Handbuch der Pflanzenkrankheiten (Handbook of plant diseases). The authors, internationally known experts in radiobiology and -biophysics, update their survey the literature on the damaging effect on plants of the ionizing radiations. In the bibliography of the original publications, the literature of the sixties and seventies dominates, which may not so much be connected with the early closing of the references and the delayed appearance of the book, as with the fact that interest in and support of research shifted in the last decade from radiobiology to other fields of science. Nor is it the authors' fault that—probably for a misinterpreted saving of space or for tradition—the book is typographically jammed, and difficult to handle.

The text of the book is thematically divided in two parts. The first six chapters which make about half of the full extent of the book can even be regarded as a self-contained monograph of general- and plant radiobiology. The first two chapters which survey the development of radiobiology and the physical bases related with the effect of ionizing radiations are followed by a description of the chemical and biochemical effects of ionizing radiations, and of the biological radiation effect in Chapters 3 and 4, Chapter 5, discussing the external and internal factors influencing the radiation injury, with its aboundant illustration from a number of original publications, gives them emphasis reasonable even from a practical point of view. Chapter 6 offers a brief survey of the theories related with the action mechanism of radiation. The last, long chapter that comprises the second half of the book treats in a clear, practicable system the wide literature on the effects of ionizing radiations on crops and their seeds, respectively, as well as on the economic and natural ecosystems, presenting quantitative data characterizing the radiation sensitiveness and radiation injury.

Most of the data contained in the book come from premediated radiobiological experiments, and the information they carry greatly contributes to both our phytopathological and radiobiological knowledge. However, as it is emphasized in the Introduction by Prof. H. Glubrecht, also an outstanding expert in the subject, this subject should not be directly mixed with the problems of radioactive contamination, which causes so much worry to the general public in connection with the nuclear weapons, the catastrophe of Chernobil or even with any peaceful use of nuclear energy. These problems are dealt with by another branch of science, radioecology. The book is primarily recommended to institutions of radiobiology, plant sanitation and plant breeding, and to those engaged in research and education in these professional fields.

J. Farkas

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